Studies on the Biosynthesis of the Mitomycin Antibiotics by *Streptomyces verticillatus*

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Summary Biosynthetic studies indicate that the mitomycins are not formed from aromatic precursors, but that [*Me*-^{14}C]-L-methionine and [*guanido*-^{14}C]-L-arginine specifically label peripheral substituents and that glucosamine is probably incorporated intact into a C_6-fragment of these antibiotics.

THE biosynthesis of the mitomycins and porfiromycin (I),[1,2] antitumour antibiotics produced by several strains of *Streptomyces*, has received relatively little attention.[3] We considered, among other hypotheses that the mitomycin skeleton could arise from two building blocks, one providing a C_7 cyclic fragment (II) and a second yielding the highly functionalized C_6 straight-chain fragment (III). This hypothesis and others were tested in feeding experiments with radioactive precursors.

Streptomyces verticillatus (ATCC 13494) was grown and replaced into a production medium essentially as described by Kirsch and Korshalla.[4] Antibiotic yield increased linearly with time to give 4—7 mg of mainly mitomycin A and B after four days. Usually 5—10 μCi ^{14}C and/or 15—30 μCi ^{3}H of radioactive precursor (spec. act. 1—2 mCi/mmol) were added on the third day. The antibiotics were isolated by extraction of the filtered broth with ethyl acetate. An aliquot portion (10—50%) was subjected to t.l.c. on silica gel plates in acetone–ligroin–n-octanol (5 : 5 : 2, run twice) (R_F values: mitomycin C, 0·17; mitomycin A, 0·34; porfiromycin, 0·40; mitomycin B, 0·45), followed by analysis with a radiochromatogram scanner. The minimum detectable incorporation of precursors into mitomycins is *ca.* 0·1%. The radiochemical purity of mitomycin A was ascertained by converting it into mitomycin C with ammonia.

The possible aromatic precursors [*alanine*-3-^{14}C]-DL-tryptophan,† [*indole*-2-^{14}C]-DL-tryptophan, [*benzene*-*U*-^{14}C]-DL-tryptophan, [*alanine*-1-^{14}C]-DL-phenylalanine, [*U*-^{14}C]-L-phenylalanine, [*alanine*-3-^{14}C]-DL-tyrosine, [*U*-^{14}C]-L-tyrosine,† and [1,6-^{14}C]-DL-shikimic acid all gave incorporations below 0·1%, as did [2-^{14}C]-DL-mevalonic acid, sodium [1-^{14}C]acetate,† sodium [2-^{14}C]propionate, sodium [^{14}C]-carbonate, sodium [^{14}C]formate, and [^{14}C]urea. All these compounds were efficiently taken up by the mycelium and parallel cultures showed incorporation of the precursors in Table 1. [1-^{14}C]-D-Xylose, [1-^{14}C]-D-arabinose, and [1-^{14}C]-L-arabinose, although taken up, gave less than 0·1% incorporation into mitomycins.

TABLE 1. *Incorporation of labelled substrates into mitomycin A, B, C, and porfiromycin by* Streptomyces verticillatus

Precursor	Incorporation (%)[a]
[*Me*-^{14}C]-L-Methionine	9·1
[*guanido*-^{14}C]-L-Arginine	4·4
[1-^{14}C]-D-Glucosamine	2·3
[*U*-^{14}C]-D-Glucose	0·5
[1-^{14}C]-D-Glucose	0·5
[2-^{14}C]-D-Glucose	0·4
[3,4-^{14}C]-D-Glucose	0·8
[6-^{14}C]-D-Glucose	0·6
[*U*-^{14}C]-D-Gluconic acid	0·7
[*U*-^{14}C]-D-Ribose	0·4
[1-^{14}C]-D-Ribose	0·4

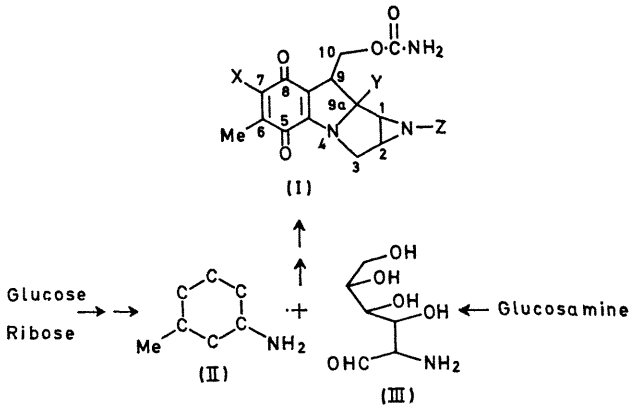
[a] Determined as described previously.[5]

The mode of incorporation of some of the compounds in Table 1 was investigated by chemical degradation and by feeding experiments with doubly labelled precursors. Removal of the peripheral substituents (*cf.* ref. 1) of the mitomycin A samples in Table 2 was achieved as follows. Four equal aliquot portions of the purified antibiotic were

† Non-incorporation of [*alanine*-3-^{14}C]-L-tryptophan, [*U*-^{14}C]-L-tyrosine, and sodium [1-^{14}C]acetate had previously been noted by Kirsch.[3]

spotted on four chromatography plates and developed in the system just given. One plate was exposed to concentrated ammonia vapour for 20 min at room temperature to remove the C-7 methoxy-group. The second plate was sprayed with 0·1N-HCl and dried with warm air to remove methanol from C-9a and C-9. The third plate was sprayed with concentrated HCl and dried for 5 min at 120° to remove the C-10 carbamyl group and methanol from C-9a and C-9, and the fourth plate was left untreated. The radioactivity of the zones at the R_F value of mitomycin A was determined by liquid scintillation counting. Radioactivity losses observed in the various samples are in Table 2.

Mitomycin A labelled from [*Me*-^{14}C]-L-methionine loses nearly all its radioactivity in the first two degradations (45·3 + 53·6 = 98·9%), confirming the conclusions of Kirsch and Korshalla[4] that only *O*-methylation but no *C*-methylation takes place in mitomycin A biosynthesis. Mitomycin A labelled from [*guanido*-^{14}C]-L-arginine loses relatively little radioactivity upon treatment with ammonia but nearly all after exposure to concentrated HCl. This indicates that radioactivity from this precursor is predominantly *incorporated* into the carbamyl group.



Structures of mitomycins and porfiromycin and hypothesis for their biogenetic origin

Compound	Substituent X	Y	Z
Mitomycin A	OMe	OMe	H
Mitomycin B	OMe	OH	Me
Mitomycin C	NH_2	OMe	H
Porfiromycin .. .. (*N*-methyl mitomycin C)	NH_2	OMe	Me

TABLE 2. *Loss of radioactivity from mitomycin A after treatment with ammonia and hydrochloric acid*

Mitomycin A labelled from	Radioactivity (disint./min) spotted on each plate	Loss of radioactivity (%) after treatment with NH_3 vapours	Dilute HCl	Concentrated HCl
[*Me*-^{14}C]-L-Methionine	$3·52 \times 10^3$	45·3	53·6	—
[*guanido*-^{14}C]-L-Arginine	$6·89 \times 10^3$	8·7	23·5	98·6
[1-^{14}C]-D-Glucosamine	$1·95 \times 10^3$	1·4	9·4	10·6
[6-^{3}H]-D-Glucosamine	$6·37 \times 10^3$	3·6	17·5	17·8
[*U*-^{14}C]-D-Glucose	$8·05 \times 10^2$	8·7	8·9	17·9
[1-^{14}C]-D-Ribose	$5·39 \times 10^2$	9·9	10·0	19·4

In contrast to [*Me*-^{14}C]-L-methionine and [*guanido*-^{14}C]-L-arginine, which label peripheral groups, glucosamine, as well as glucose and ribose, can be expected to act as precursors for the carbon skeleton of the mitomycins. In experiments with the aminohexose [1-^{14}C,6-^{3}H]-D-glucosamine gave 1·9% incorporation with a tritium retention in the mitomycins of 91%, and [1-^{14}C,1-^{3}H]-D-glucosamine gave 2·7% incorporation with 78% retention of tritium. Carbon-14 and tritium incorporated from [1-^{14}C,6-^{3}H]-D-glucosamine were predominantly located in hydrolytically stable positions (Table 2). Glucosamine does not seem to provide label to the C_7 unit since acetic acid obtained by Kuhn–Roth oxidation of purified mitomycin A (78·0 mg; $5·86 \times 10^3$ disint./min) labelled from [1-^{14}C]-D-glucosamine contained essentially no radioactivity (0·4% of the specific radioactivity of mitomycin A). These results suggest that glucosamine predominantly acts in an intact form as a precursor for the C-6 unit.

Mitomycin A labelled from glucose or ribose contains radioactivity predominantly in the carbon skeleton (Table 2). Ribose provides label to the C-7 unit as shown by Kuhn–Roth oxidation of purified mitomycin A (100 mg; $1·13 \times 10^4$ disint./min) obtained from [*U*-^{14}C]-D-ribose, which yielded acetic acid with 13·3% of the specific radioactivity of the antibiotic. These data and other evidence, to be presented later, suggest that the C-7 unit of the mitomycins can be formed from glucose and/or ribose possibly *via* a heptose intermediate. An analogy for the pathway envisaged for the C-7 unit can be seen in the biosynthesis of laminitol, a *C*-methyl-inositol occurring in algae, in which glucose has been shown to act as a precursor *via* its conversion into a heptose.[6]

Data which supplement and extend our own have been obtained recently by Vining and his co-workers in their studies on the biosynthesis of mitomycin C.

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