Mitomycin Biosynthesis by *Streptomyces verticillatus*. Incorporation of the Amino-group of D-[^{15}N]Glucosamine into the Aziridine Ring of Mitomycin B

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Summary It was demonstrated that among a number of hexosamines tested as precursors of the mitomycin antibiotics, D-glucosamine was most efficiently incorporated, and that it can provide the nitrogen atom of the aziridine ring of mitomycin B.

PREVIOUS investigations into the biosynthesis of the mitomycin antibiotics (I) by *Streptomyces verticillatus* have shown that D-glucosamine is an efficient precursor and that it is most likely incorporated as an intact unit.[1] It is assumed that C-1 and -6 of this amino-sugar become C-3 and -10, respectively, of the mitomycins and that the nitrogen atom of the aziridine ring originates from the amino-group of the aminohexose. Experiments to test this latter assumption and experiments probing the role of other hexosamines in mitomycin biosynthesis are reported.

The feeding to *S. verticillatus* of the compounds listed in Table 1, the isolation of the mitomycins produced, and the determination of the precursor incorporation were carried out by methods similar to those described previously.[1] A double replacement feeding technique was used for the

administration of the samples of D-[1-^{14}C,^{15}N]glucosamine† (Table 2) to obtain high specific incorporation. Mitomycin B produced in the latter series of experiments was subjected to m.s. analysis for the determination of the incorporation and the distribution of the ^{15}N label. Mitomycin B, (I), gives a strong fragment ion at *m/e* 70 containing the aziridine nitrogen atom. This ion has the composition C_4H_8N, (II), and arises as indicated by the slashed line in (I).[3] Since

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

Precursor	Quantity fed (μmol)	Radio-activity fed (d.p.m.)	Incor-poration (%)
D-[1-^{14}C]glucosamine ..	2·9	2·1 × 10^7	2·3
D-[1-^{14}C]galactosamine ..	2·9	2·1 × 10^7	1·8
D-[1-^{14}C]mannosamine ..	4·7	1·8 × 10^7	0·6
D-[1-^{14}C]glucosamine ..	20·0	4·3 × 10^6	3·0
L-[2-^{14}C]glucosamine[a] ..	20·5	4·6 × 10^6	<0·01

[a] Synthesized as described in ref. 2.

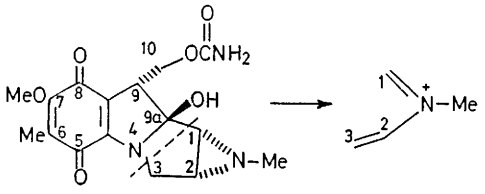
impurities caused variable ratios of the peaks *m/e* 70 and 71 in low resolution spectra, the ions $C_4H_8{}^{14}N$ and $C_4H_8{}^{15}N$ were identified by high resolution mass spectrometry (direct inlet probe, 180°, 70 eV, accuracy: 3 millimass units). The intensities of these ions were measured after scanning them at least ten times. The intensities of further fragment ions were measured in low-resolution spectra (direct inlet probe, 180°, 70 eV) of labelled and non-labelled reference mitomycin B. Analysed in both experiments was an ion cluster at *m/e* 288, representing the ion $[M - HNCO]^{+\cdot}$ which arises by fragmentation of the carbamoyl group and an ion cluster at *m/e* 331, representing the ion $[M - H_2O]^{+\cdot}$. Values for the specific incorporation of ^{15}N into the different nitrogen atoms of mitomycin B were calculated from the measured intensities. These values and the values for the specific incorporation of the ^{14}C reference label are given in Table 2.

TABLE 2. *Specific incorporation of* D-[1-^{14}C, ^{15}N]*glucosamine into mitomycin B by* Streptomyces verticillatus

	Amount of D-[1-^{14}C,^{15}N]-glucosamine fed (μmol)	(d.p.m.)	Specific incorporation (%)[a] of ^{14}C	of ^{15}N into the aziridine ring nitrogen	the carbamoyl nitrogen	the "indolic" nitrogen
Experiment A	239·2	1·3 × 10^7	37	44	18	5
Experiment B	465·0	1·1 × 10^7	83	77	24	10

[a] The relative error is estimated to be ±10%.

It is apparent from the results of the feeding experiments with hexosamines (Table 1) that D-glucosamine is the most efficient mitomycin precursor tested. D-Galactosamine and D-mannosamine probably are incorporated after their conversion into D-glucosamine. This assumption is supported for D-galactosamine by results of competition feeding experiments.[4] D-Glucosamine occurs in a variety of antibiotics[5] but also its L-isomer is found in nature. For instance the *N*-methyl derivative of L-glucosamine is a constituent of the *Streptomyces* antibiotic streptomycin and this moiety is most likely derived biosynthetically from D-glucosamine.[6] To test for a possible involvement of L-glucosamine in mitomycin biosynthesis this compound



(I) Mitomycin B (II) Fragment ion C_4H_8N

was included in the feeding experiments. Apparently free L-glucosamine does not function in this pathway even though uptake by the mycelium (7%) could be demonstrated.

The data on the specific incorporation of ^{14}C and of ^{15}N in the two experiments with D-[1-^{14}C,^{15}N]glucosamine show that the ^{14}C incorporation into mitomycin B and the ^{15}N incorporation into the aziridine ring are similar and nearly parallel the amount of the precursor added. This suggests that both isotopes are predominantly incorporated without separation and it is concluded that D-glucosamine can provide the nitrogen atom of the aziridine ring. Some breakdown of D-glucosamine seems to take place, however, since a fraction of the ^{15}N is clearly incorporated also into the carbamoyl group and into the "indolic" nitrogen of mitomycin B.

It appears that C-2 of D-glucosamine is incorporated with inversion of configuration. This follows from the precursor configuration and the absolute configuration of C-2 of the mitomycins which, as deduced from *X*-ray data by Tulinsky and Van der Hende,[7] is *R*. It is not known at what stage the inversion occurs but it can be excluded that L-glucosamine and D-mannosamine, both of which have *S* configuration at C-2, participate as the free compounds. The possibility remains that derivatives of these amino-sugars such as nucleotides, which cannot be formed directly from the free amino-sugars, are intermediates in mitomycin biosynthesis.

We thank W. Perry for mass spectral determinations, Dr. S. Wakaki, Kyowa Hakko Kogoyo Co. Ltd., for samples of mitomycins, and the N.I.H. for financial support.

(*Received*, 30*th October* 1972; *Com.* 1840.)

† D-[^{15}N]glucosamine (95 atom % enrichment of ^{15}N) was synthesized[2] and mixed with D-[1-^{14}C]glucosamine, which served as a reference label.

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