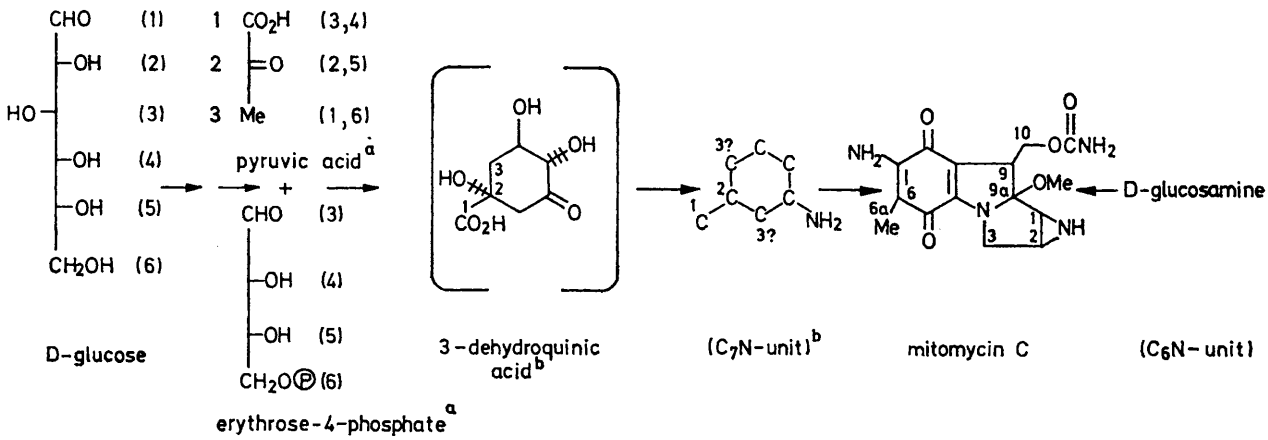
Pyruvic Acid and D-Glucose as Precursors in Mitomycin Biosynthesis by *Streptomyces verticillatus*

By ULFERT HORNEMANN,* JAMES P. KEHRER and JAMES H. EGGERT
(*Department of Medicinal Chemistry and Pharmacognosy, Purdue University, West Lafayette, Indiana* 47906)

Summary. Feeding experiments and degradations have shown that [1-^{14}C]pyruvic acid and D-[3,4-^{14}C]glucose specifically label C-6a of mitomycin C, and that [2-^{14}C]-pyruvic acid and D-[2-^{14}C]glucose specifically label C-6 of this antibiotic.

RECENT studies on the biosynthesis of the mitomycin antibiotics have indicated that D-glucosamine provides C-1, C-2, C-3, C-9, C-9a, and C-10 and the nitrogen atom of the aziridine ring,[1,2] leading to the assumption that the carbon-nitrogen skeleton of these antibiotics is built up by the joining of a C_6N and a C_7N unit. It has been suggested

Suggested pathway of mitomycin biosynthesis involving pyruvic acid, D-glucose and D-glucosamine.



[a] Numbers in brackets refer to those of D-glucose. [b] Numbers refer to those of pyruvic acid.

that the C_7N unit could arise from a C_7 sugar,[2,3,4] however, Bezanson and Vining[5] have reported feeding experiments with D-[1-^{14}C]-, -[2-^{14}C]-, and -[6-^{14}C]glucose, which afforded specific labelling of C-6 and C-6a of mitomycin C, yet showed an unexpectedly large difference in the labelling of these two atoms by D-[1-^{14}C]- and -[2-^{14}C]glucose (4·42% versus 14·5% in C-6 + C-6a), which could not be explained readily by the assumed involvement of a heptulose in mitomycin biosynthesis.

In order to suppress the likely incorporation of D-glucose into the C_6N unit and thus reveal more clearly a possible difference in the labelling of C-6 + C-6a by D-[1-^{14}C]- and -[2-^{14}C]glucose we administered[3] D-[1-^{14}C]-, -[2-^{14}C]- and -[6-^{14}C]glucose together with a large excess of unlabelled D-glucosamine. The results of the feeding experiments with these glucose species and of the degradations[5] of the mitomycin C samples obtained (Table) suggest that it is likely that C-3 rather than C-1 of D-glucose would provide the methyl group† and that this hexose is incorporated into C-6 and C-6a *via* phosphoenolpyruvate. This assumption was tested by feeding D-[3,4-^{14}C]glucose and the specifically labelled samples of pyruvic acid listed in the Table. While the locations of the label from [3-^{14}C]pyruvic acid and D-[6-^{14}C]glucose and the majority of the label from D-[1-^{14}C]glucose remain to be established the results indeed suggest an involvement of phosphoenolpyruvate in mitomycin biosynthesis. Since previous experiments[3,5] showed that shikimic acid is not a mitomycin precursor it is suggested that 3-dehydroquinic acid may be acting as an intermediate, however other possible intermediates arising from pyruvate cannot be excluded.

TABLE. Mode of incorporation of D-glucose and pyruvic acid into mitomycins by *S. verticillatus*.

Precursor[a]	Quantity fed /μ mol	Radio-activity fed /dpm	Incorporation[b] into mitomycins A, B, C, and porfiromycin[c] /%	Specific radio-activity of mitomycin C before degradation /dpm mmol^{-1}	Radio-activity of mitomycin C remaining in tetra-acetyl-mitomycinone derivative[5] /%	Radio-activity of mitomycin C residing in C-6+C-6a /%	Radio-activity of C-6+C-6a residing in C-6a /%	Radio-activity of C-6+C-6a residing in C-6 /%
D-[1-^{14}C]glucose	24·1	$4·0 \times 10^7$	0·7	$7·3 \times 10^4$	68	4·6	44·0	19·0
D-[2-^{14}C]glucose	21·7	$3·1 \times 10^7$	0·8	$7·1 \times 10^4$	63	28·2	21·0	75·5
D-[3,4-^{14}C]glucose	18·6	$2·1 \times 10^7$	0·3	$8·0 \times 10^4$	74	22·6	61·2	9·9
D-[6-^{14}C]glucose	23·2	$4·3 \times 10^7$	0·8	$1·0 \times 10^5$	76	0·4	—	—
[1-^{14}C]pyruvic acid	19·4	$3·9 \times 10^7$	0·2	$1·0 \times 10^5$	33	27·4	104·7	0
[2-^{14}C]pyruvic acid	20·7	$4·0 \times 10^7$	0·2	$6·3 \times 10^4$	68	40·0	6·5	75·7
[3-^{14}C]pyruvic acid	20·3	$3·8 \times 10^7$	0·5	$1·2 \times 10^5$	61	2·0	35·7	60·3

[a] Administered together with 465 μmol of unlabelled D-glucosamine to suppress incorporation into the C_6N unit.
[b] Total radioactivity in mitomycins/total radioactivity administered × 100.
[c] The variations are assumed to be related to differences in the amounts of mitomycins produced in individual cultures (normal range: 4–6 mg/100 ml culture medium[3]).

We thank the National Institute of Health for support and Dr. I. Pachter (Bristol Laboratories) for a gift of mitomycin C.

(*Received, 20th August* 1974; *Com.* 1081.)

† A contribution of C-3 of D-glucose to the labelling of the methyl group was also considered among other possibilities by Bezanson and Vining.[5]

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