Role of D-[4-^{14}C]Erythrose and [3-^{14}C]Pyruvate in the Biosynthesis of the *meta*-C–C_6–N Unit of the Mitomycin Antibiotics in *Streptomyces verticillatus*

By ULFERT HORNEMANN,* JAMES H EGGERT, and DANIEL P HONOR

(*Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana* 47907)

Summary D-[4-^{14}C]Erythrose but not [3-^{14}C]pyruvate labels C-7 of the mitomycins significantly, while [7-^{14}C]-3-dehydroquinic acid and D-[1 6-$^{14}C_2$]shikimic acid methyl ester are not incorporated leading to the suggestion that 4-amino-3,4-dideoxy-D-arabinoheptulosonic acid 7-phosphate, or a close relative, may act as an intermediate in the formation of the *meta*-C–C_6–N unit of these antibiotics

BIOSYNTHETIC studies with carbon-14-labelled pyruvate and glucose [1] with carbon-13-labelled glucoses,[2,3] and glucose and glyceric acid[4] as well as with other precursors have indicated that the mitomycins,[1] geldanamycin,[2] pactamycin,[3] and the rifamycins,[4] respectively, share the presence of *meta*-C–C_6–N units (C_7N units) which presumably arise from an early intermediate of the shikimic acid pathway 3-Dehydroquinic acid (3DHQ), which in bacteria is formed *via* 3-deoxy-D-arabinoheptulosonic acid 7-phosphate (DAHP) from phosphoenolpyruvate and erythrose-4-phosphate, has been postulated as a likely intermediate in each case The work reported herein shows for the first time the specific incorporation of D-[4-^{14}C]erythrose into the *meta*-C–C_6–N unit of a member of this group of antibiotics and leads us to suggest that 4-amino-4-deoxy DAHP or a closely related compound may act as an intermediate in the formation of *meta*-C–C_6–N units

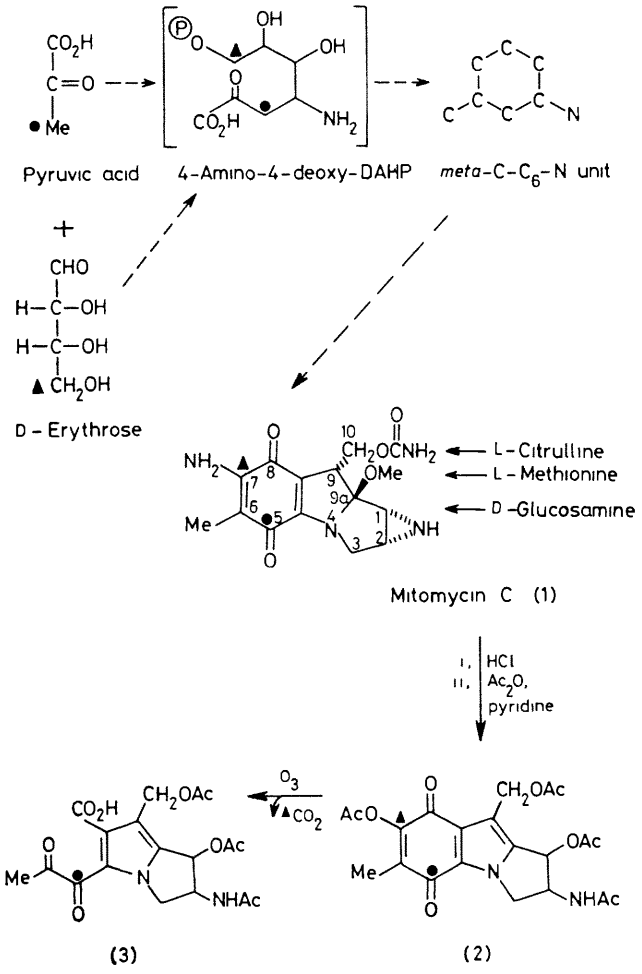
D-[4-^{14}C]Erythrose, prepared from D-[6-^{14}C]glucose, and sodium [3-^{14}C]pyruvate were administered to *S verticillatus* (ATCC 13495) as previously described [5] Mitomycin A was isolated and converted into mitomycin C[5] which was recrystallized with carrier and degraded[6] as outlined in the Table and the Scheme [3-^{14}C]Pyruvate incorporation†

† Pyruvate can be converted in *S verticillatus* into phosphoenolpyruvate by an inducible pyruvate phosphate dikinase (K L Redman and U Hornemann Abstracts Joint Central–Great Lakes Regional Meeting American Chemical Society, Indianapolis, Indiana May 24–26th 1978, Biol 16)

TABLE. Mode of incorporation of [3-^{14}C]pyruvate and D-[4-^{14}C]erythrose into mitomycins by *S. verticillatus*

	Sodium [3-^{14}C]pyruvate	D-[4-^{14}C]erythrose
Quantity fed (mg)	2·2	7·3
Radioactivity fed (d.p.m.)	$4{\cdot}36 \times 10^7$	$4{\cdot}93 \times 10^7$
Incorporation into mitomycins A, B, C, and porfiromycin (%)[a]	0·2	0·8
Specific radioactivity of mitomycin C		
1st recryst. (d.p.m./mmol)	$6{\cdot}9 \times 10^4$	$1{\cdot}91 \times 10^5$
2nd recryst. (d.p.m./mmol)	$6{\cdot}9 \times 10^4$	$1{\cdot}83 \times 10^5$
Specific radioactivity of tetra-acetylmitomycinone derivative (**2**)		
1st recryst. (d.p.m./mmol)	$4{\cdot}98 \times 10^4$	$1{\cdot}99 \times 10^5$
2nd recryst. (d.p.m./mmol)	$4{\cdot}94 \times 10^4$	$1{\cdot}78 \times 10^5$
Radioactivity of mitomycin C remaining in tetra-acetylmitomycinone derivative (%)[b]	72	97
Specific radioactivity of tetra-acetylmitomycinone ozonolysis product (**3**)		
1st pptn. (d.p.m./mmol)	$4{\cdot}47 \times 10^4$	$1{\cdot}08 \times 10^5$
2nd pptn. (d.p.m./mmol)	$4{\cdot}48 \times 10^4$	$1{\cdot}10 \times 10^5$
Radioactivity of mitomycin C remaining in ozonolysis product (%)[b]	65	60
Radioactivity of mitomycin C residing at C-7 (%)[b]	7	37

[a] Total radioactivity in mitomycins/total radioactivity administered $\times 100$. [b] Calculated with the values obtained in respective second repurification step.



SCHEME. Precursors of mitomycin biosynthesis in *S. verticillatus*.

and the extent of labelling of the peripheral $-OCONH_2$ and –OMe groups (28%) were comparable with previous results.[1] Only 7% of the label of this precursor resides at C-7, while, as shown previously,[1] [1-^{14}C]- and [2-^{14}C]-pyruvate provide 27 and 25%, respectively, of their label to C-6a and C-6. Thus, making the plausible assumption that the methyl group is not detached upon the incorporation of [3-^{14}C]pyruvate, it is highly likely that a major portion of its label resides at C-5. D-[4-^{14}C]Erythrose is efficiently incorporated‡ and it does not significantly label the peripheral groups. Nearly 40% of the label resides at C-7. This result and the mode of incorporation of pyruvate suggest that both precursors are incorporated into the quinone unit (*meta*-C–C_6–N unit) of the mitomycins essentially as shown in the Scheme. Clearly a large amount of the label from D-[4-^{14}C]erythrose must reside in other positions and C-10 (labelled after conversion of D-erythrose into the mitomycin precursor D-glucosamine[7] *via* D-glucose) and C-5 (upon formation of [3-^{14}C]pyruvate) can be suggested as likely locations.

[7-^{14}C]-3-Dehydroquinic acid (0·7 mg, $9{\cdot}9 \times 10^6$ d.p.m.), prepared enzymatically using sodium [1-^{14}C]phosphoenolpyruvate (New England Nuclear Co.), and D-[1,6-$^{14}C_2$]-shikimic acid methyl ester (7 mg, $1{\cdot}9 \times 10^7$ d.p.m.) were administered as above and the isolated mitomycins were analysed by radiochromatogram scanning which revealed less than 0·01% incorporation. Detailed uptake studies for these precursors were not carried out, but in conjunction with the reported[8] non-incorporation of shikimic acid, for which uptake by the mycelium has been ascertained,[8] it is considered unlikely that 3DHQ actually plays a role in mitomycin biosynthesis. Therefore the presently unknown compound 4-amino-4-deoxy DAHP, or a close relative, which could be derived from 5-dehydro DAHP, a postulated intermediate in 3DHQ formation in bacteria,[9] is suggested to be an early precursor in the formation of the *meta*-C–C_6–N unit of the mitomycins and of the *meta*-C–C_6–N unit of the other antibiotics containing them.

We thank Dr. K. M. Herrmann and M. Poling, Purdue

‡ No information is available at present on the conversion of erythrose into its 4-phosphate which is presumably required before it can be incorporated.

University for preparative help, Dr. M. A. Abd-El Rahman, Alexandria University, Dr. S. Singaram, Purdue University, Dr. S. Wakaki, Kyowa Hakko Kokyo, Tokyo, and Dr. I. Pachter, Bristol Laboratories, Syracuse, for gifts of chemicals, and the National Cancer Institute, D.H.E.W., for financial support.

(*Received, 24th July* 1979; *Com.* 806.)

[1] U. Hornemann, J. P. Kehrer, and J. H. Eggert, *J.C.S. Chem. Comm.*, 1974, 1045.
[2] A. Haber, R. D. Johnson, and K. L. Rinehart, Jr., *J. Amer. Chem. Soc.*, 1977, **99**, 3541.
[3] D. D. Weller and K. L. Rinehart, Jr., *J. Amer. Chem. Soc.*, 1978, **100**, 6757.
[4] R. J. White and E. Martinelli, *F.E.B.S. Letters*, 1974, **49**, 233.
[5] U. Hornemann and J. C. Cloyd, *Chem. Comm.*, 1971, 301.
[6] C. L. Stevens, K. G. Taylor, M. E. Munk, W. S. Marshall, K. Noll, G. D. Shah, L. G. Shah, and K. Uzu, *J. Medicin. Chem.*, 1965, **8**, 1.
[7] U. Hornemann, J. P. Kehrer, C. S. Nunez, and R. L. Ranieri, *J. Amer. Chem. Soc.*, 1974, **96**, 320.
[8] G. S. Bezanson and L. C. Vining, *Canad. J. Biochem.*, 1971, **49**, 911.
[9] U. S. Maitra and D. B. Sprinson, *J. Biol. Chem.*, 1978, **253**, 5426.