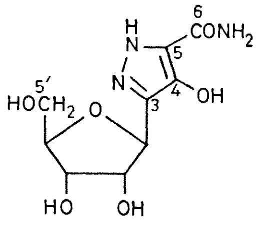
The Biosynthesis of Pyrazofurin and Formycin

By J. Grant Buchanan,* Michael R. Hamblin, Gulshan R. Sood, and Richard H. Wightman*
(*Department of Chemistry, Heriot-Watt University, Edinburgh* EH14 4AS)

Summary Evidence is presented that D-ribose and L-glutamate are the principal biosynthetic precursors of pyrazofurin (**1**) and formycin (**2**).

The C-nucleoside antibiotic pyrazofurin (pyrazomycin)[1] (**1**), a metabolite of *Streptomyces candidus* possessing both anti-viral and anti-tumour activity,[1] contains a pyrazole ring, a very rare feature amongst natural products. We now report experiments casting light on the biosynthesis of pyrazofurin (**1**), and of the structurally related antibiotic formycin[1] (**2**).



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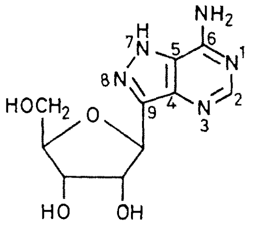

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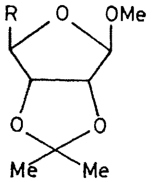
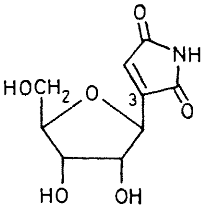


(3) R = CH$_2$OH
(4) R = CHO
(5) R = CHDOH



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The results of experiments with various potential precursors of pyrazofurin in labelled form are summarised in the Table. The high incorporation of [1-^{14}C]ribose, considerably greater than that of [1-^{14}C]glucose, strongly implies that ribose is a direct precursor of the ribofuranosyl residue of pyrazofurin. This was confirmed by means of an experiment using deuterium labelling. Oxidation of methyl 2,3-*O*-isopropylidene-β-D-ribofuranoside[2] (**3**) with chromium trioxide–pyridine, and reduction of the resultant aldehyde[3] (**4**) with sodium borodeuteride gave the labelled ribofuranoside (**5**); the introduction of one deuterium atom at C-5 was confirmed by ^{1}H n.m.r. spectroscopy. The deuteriated ribose (0·64 g) produced by acid hydrolysis of (**5**) was administered, mixed with [1-^{14}C]ribose, to *Streptomyces candidus*. The pyrazofurin subsequently isolated and recrystallised (0·15 g) had specific activity 7·9% that of the ribose fed. The proton-decoupled ^{2}H n.m.r. spectrum (55·3 MHz, H_2O) of this pyrazofurin showed one signal at δ 3·82.† The ^{1}H n.m.r. spectrum of pyrazofurin under similar conditions (360 MHz, D_2O) is completely resolved, and the protons of the 5′-position appear, as the AB part of an ABX system, centred at δ 3·73.† The intact and specific incorporation of ribose is thus demonstrated.

With regard to the origins of the pyrazole ring and amide carbon, the negligible incorporation of label from hydrogen carbonate (see Table) indicates that the amide carbon is

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† Chemical shifts were measured relative to HOD (taken as δ 4·70) as internal standard; thus some deviations between spectra are to be expected.

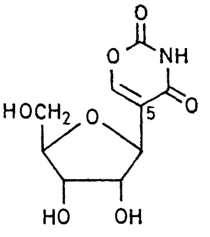
TABLE Incorporation of ^{14}C-labelled metabolites into pyrazofurin

Labelled compound[a]	Activity administered/μCi	Pyrazofurin isolated (mg)	Specific activity /d min^{-1} μmol^{-1}	% Incorporation
[1-^{14}C]-D-ribose	50	106	16280	6 0
[1-^{14}C]-D-glucose	45	130	2570	1 3
[^{14}C]NaHCO$_3$	1000	104	767	0 014
[1-^{14}C]glycine[b]	100	80	560	0 08
[2-^{14}C]glycine[b]	100	123	3335	0 7
[U-^{14}C]-L-glutamate[c]	40	197	1267	1 1
[1-^{14}C]-L-glutamate[c]	17	122	290	0 37
[U-^{14}C]-L-lysine	50	121	168	0 07

[a] Compounds were administered to cultures of *S candidus* grown for 2 days in a medium of glycerol (10 g), soy peptone (5 g), Ca(NO$_3$)$_2$ 4H$_2$O (2 g), and distiller's solubles (10 g/l), adjusted to pH 6 8, pyrazofurin was isolated from the filtrate after a further 5 days [b] Experiments carried out simultaneously in parallel [c] Administered admixed with unlabelled material (0 1 g), specific incorporations 0·9% for [U-^{14}C] label, 0 53% for [1-^{14}C]

not introduced by carboxylation, as might have been postulated by analogy with purine biosynthesis[4] Similarly, the substantially larger incorporation of [2-^{14}C]glycine compared with [1-^{14}C]glycine strongly argues against glycine as an intact precursor, standard pathways of metabolism[5] can lead to incorporation of the methylene position, but not the carboxy carbon, of glycine into the Krebs cycle However, the results of the experiments with labelled glutamic acid strongly suggest that it, or α-ketoglutarate, is a specific precursor The incorporation of [1-^{14}C]glutamate is predictably less than that of uniformly labelled material, owing to partial metabolism of glutamate by transamination and the Krebs cycle, any metabolism of glutamate in this way leads to direct loss of C-1 as CO$_2$ As a consequence of these experiments, and experiments on formycin discussed below, we postulate that C-3 to C-6 of pyrazofurin are derived from C-4 to C-1 of glutamate or α-ketoglutrate

Structural analogy strongly suggests that the biosynthesis of formycin (**2**) is closely related to that of pyrazofurin (**1**) Ochi and co-workers have recently reported[6] that feeding of [U-^{13}C]glutamate produced formycin showing enhanced ^{13}C n m r signals for C-9, C-4, C-5, and C-2, [see (**2**)] with C-6 being labelled by [U-^{13}C]lysine In the light of these somewhat surprising results, we have re-examined the ^{13}C n m r spectrum of formycin under the conditions used by Ochi,[6]‡ and we have found that the lowest-field signal (155 2 p p m in our measurements) must be assigned to C-6, since it remains a singlet on off-resonance decoupling, whilst the next-lowest signal (148 9 p p m) is clearly that of C-2, since it becomes a doublet on off-resonance decoupling These assignments reverse those of Ochi[6] Thus Ochi's findings can be interpreted as glutamate giving rise to the four *contiguous* positions, 9, 4, 5, and 6 in formycin Since significant incorporation of [1-^{14}C]glutamate was also reported,[6] these positions in formycin must be derived from C-4 to C-1 of glutamate Furthermore, we have administered [U-^{14}C]lysine to *S candidus*, and found only slight incorporation into pyrazofurin §



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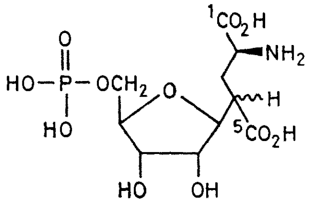

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The maleimide ring in showdomycin (**6**) has been shown to be derived from glutamate in an unsymmetrical manner, C-3 of showdomycin being derived from C-4 of glutamate,[7] the heterocyclic ring of oxazinomycin (**7**) is also derived from glutamate, with C-5 originating from C-4 of glutamate[8] It is thus tempting to suggest that the biosynthesis of all the C-nucleoside antibiotics involves a common intermediate such as (**8**), derived from ribose (as phosphoribosyl pyrophosphate) and glutamate (or α-ketoglutarate), with loss of C-1 in formation of showdomycin and oxazinomycin, and loss of C-5 in pyrazomycin and formycin biosynthesis

We thank Dr R L Hamill (Eli Lilly, Indianapolis) for cultures of *S candidus* and a sample of pyrazofurin, the S R C for financial support and access to the high-field n m r facility at Edinburgh University, directed by Dr I H Sadler, and Dr Kartar Singh, Principal, Ramjas College, University of Delhi, India, for granting leave of absence to G R S

(*Received*, 7*th July* 1980, *Com* 737)

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‡ These conditions restrict tautomerism and permit resolution of signals of C-2 and C-6, in contrast to earlier studies (T R Krugh, *J Am Chem Soc*, 1973, **95**, 4761, M-T Chenon, R P Panzica, J C Smith, R J Pugmire, D M Grant, and L B Townsend, *ibid*, 1976, **98**, 4736)

§ The mechanism by which lysine labels C-2 of formycin is unclear

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