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STEREOCHEMISTRY ASSOCIATED WITH THE CONVERSION OF C-3 OF SERINE THROUGH METHYLENETETRAHYDROFOLATE AND METHENYLTETRAHYDROFOLATE IN THE BIOSYNTHESIS OF TUBERIN.

Karl M. Cable and Richard B. Herbert*

Department of Organic Chemistry, The University, Leeds LS2 9JT.

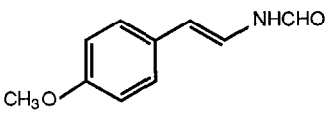
Vanessa Bertram and Douglas W. Young

School of Chemistry and Molecular Sciences, University of Sussex, Falmer, Brighton BN1 9QJ.

Summary: Results of feeding experiments in Streptomyces amakusaensis with (2S, 3R)-[3-$^{2}H_{1}$]- and (2S, 3S)-[2,3-$^{2}H_{2}$]-serine show that the N-formyl group in tuberin (1) is formed from C-3 of serine via tetrahydrofolate-linked intermediates with retention of the 3-pro-S proton; retention is only partially stereospecific. The relevance to tetrahydrofolate metabolism is discussed.

Tuberin (1) is a metabolite produced by Streptomyces amakusaensis.[1] It is biosynthesised in part from tyrosine[2,3] and the O-methyl and N-formyl groups are labelled by [2-^{14}C]-glycine through C_1-tetrahydrofolate intermediates.[2,4] 5,10-Methylenetetrahydrofolate (2) is formed by condensation of tetrahydrofolate with a C_1 unit (formaldehyde equivalent) which may arise from glycine by way of the glycine cleavage system [C-2 of glycine becomes C-11 of (2)]; 5,10-methylenetetrahydrofolate is then metabolized to provide C_1 units at the methanol level of oxidation, e.g. the O-methyl group in (1), or at the formic acid level of oxidation, e.g. the N-formyl group in (1).[5] The latter metabolic process involves dehydrogenation of (2) to give 5,10-methenyltetrahydrofolate (3), a process which in liver results in stereospecific removal of the 11-pro-R hydrogen.[6] It has been shown that overall in the conversion of glycine (4) into the N-formyl group of (1) the 2-pro-S hydrogen in the amino acid (4) is retained with (partial) stereospecificity, i.e. the 2-pro-R hydrogen in glycine becomes the 11-pro-R hydrogen in (2).[4] The same conclusion was reached in related but different experiments with Escherichia coli.[7] C-3 of serine may also act, via tetrahydrofolate linked intermediates, as a C_1 source; we report here stereochemical results relating to the incorporation of serine (5) into tuberin (1).

Samples of (2S, 3R)-[3-$^{2}H_{1}$]serine and (2S, 3S)-[2,3-$^{2}H_{2}$]serine were prepared,[8] mixed with [3-^{14}C]-serine, and administered to S. amakusaensis. The serine precursors were well



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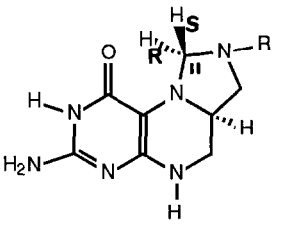

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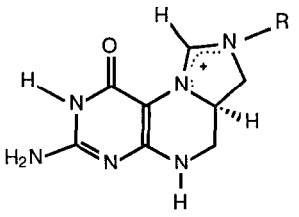

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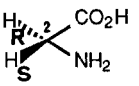

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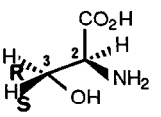

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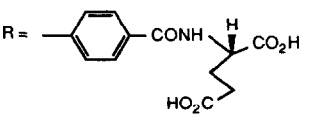

incorporated into tuberin (1) (Table). ^{2}H N.m.r. analysis using the resonance for the O-methyl group of (1) as an internal reference (both precursors are expected to label this group similarly through metabolism via 5-methyltetrahydrofolate and methionine) show that deuterium in the (3S)-position of serine is preferentially retained in the N-formyl group of tuberin (1). (Figure) If the dehydrogenase is specific for removal of the 11-pro-R hydrogen, as was found for the liver enzyme,[6] then our result implies that the 3-pro-R hydrogen of serine becomes the 11-pro-R hydrogen in 5,10-methylenetetrahydrofolate (2).

The transfer of C-3 of serine to tetrahydrofolate which generates (2) (and glycine) is catalysed by serine hydroxymethyltransferase (SHMT).[5] It has been clearly demonstrated using isolated enzymes that in the transfer of C-3 of serine to tetrahydrofolate the 3-pro-R proton becomes the 11-pro-R proton in (2), but the enzymic reaction proceeds with only partial stereochemical control.[6] Our results with tuberin then corroborate the stereochemical results observed with isolated SHMT from liver and also the incomplete stereochemical control in this process (Table). It is notable that this lack of control is also observed in vivo in E. Coli .[9] When chirally deuteriated glycine was used[4] as a tuberin precursor some loss of stereochemical control was also observed, again presumably due to the action of SHMT, but appropriately this loss of stereochemical identity was not nearly so marked.

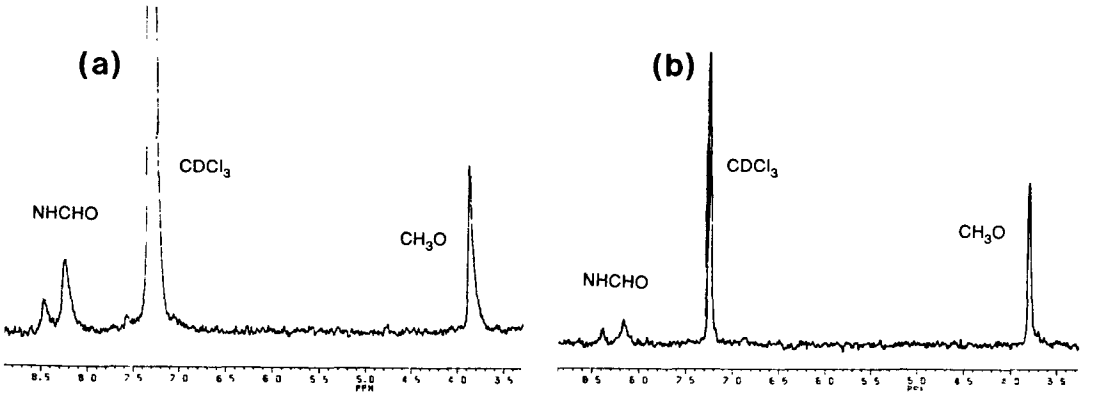

Figure ^{2}H N.m.r. spectra of tuberin (1) in $CHCl_3$ at 61.4 MHz: (a) material derived from (2S, 3S)-[2,3-$^{2}H_2$]serine; (b) material derived from (2S, 3R)-[3-$^{2}H_1$]serine. Two signals are observed for the N-formyl group of (1) due to the presence of two amide rotamers.

Our results with glycine and serine are based on the reasonable assumption that the stereochemistry found for the conversion of (2) into (3) using liver enzyme also applies in microbial systems. We are currently seeking to check this using (2) chirally labelled on C-11. Poor incorporations of [11-^{14}C]methylenetetrahydrofolate into tuberin in intact cells directs us towards the use of cell-free preparations.

Table. Incorporation of (2S)-[^{2}H, 3-^{14}C]serine into tuberin (1).

Expt.[a]	Serine Precursor[b]	^{14}C: Specific Incorporation %	^{2}H: Ratio CHO/OMe
1	(2S, 3R)-[3-$^{2}H_1$]	10.7	0.5
	(2S, 3S)-[2,3-$^{2}H_2$][c]	7.8	0.95
2	(2S, 3R)-[3-$^{2}H_1$]	3.1	0.3
	(2S, 3S)-[2,3-$^{2}H_2$][c]	3.2	0.95

a: The two samples in each experiment were fed in parallel to S. amakusaensis cultures in four aliquots over 2½ days after tuberin production had commenced.

b: Each sample contained (2S)-[3-^{14}C]serine.

c: Deuterium label at C-2 in this precursor is retained in the (2R)-position of glycine generated by the action of SHMT;[5] the deuterium would be removed from any of this glycine which is incorporated into the N-formyl group of tuberin[4] but be retained in the O-methyl group. Thus the ratios obtained for this precursor may be (slight) underestimates.

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