Biosynthesis of Tuberin from Tyrosine and Glycine

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The *N*-formyl and *O*-methyl groups of tuberin (**8**) are labelled efficiently by [2-^{14}C]glycine but the *N*-formyl group of (**8**) is not labelled by various other precursors notably [^{14}C]formate and [*Me*-^{14}C]methionine [the latter precursor labels the *O*-methyl group of (**8**) very efficiently]; L-tyrosine serves as a precursor for the remaining carbons in tuberin (**8**) but its incorporation is not completely intact, and DL-[2-^{14}C]-*threo*-3-hydroxytyrosine (**5**) is incorporated into (**8**) apparently only *via* glycine.

Studies of the biosynthesis of the intriguing microbial isonitriles, (**1**) (*Trichoderma hamatum*)[1] and xanthocillin-X (**2**) (*Penicillium notatum*),[2] have shown that the carbon skeleton of each metabolite is elaborated from tyrosine. Critically

though, the origin of the isonitrile atoms has remained unknown. A strikingly similar structure to that of xanthocillin-X (**2**) is found in the simple metabolite, tuberin (**8**), which is produced by *Streptomyces amakusaensis*.[3] Results reported here define the origins of (**8**) and have a bearing on the biosynthesis of (**2**); preliminary and parallel results on the biosynthesis of xanthocillin-X dimethyl ether (**3**) in *Aspergillus clavatus* indicate that the origin of the isonitrile carbon atoms in (**3**) may be the same as that of the *N*-formyl group in tuberin (**8**).[4]

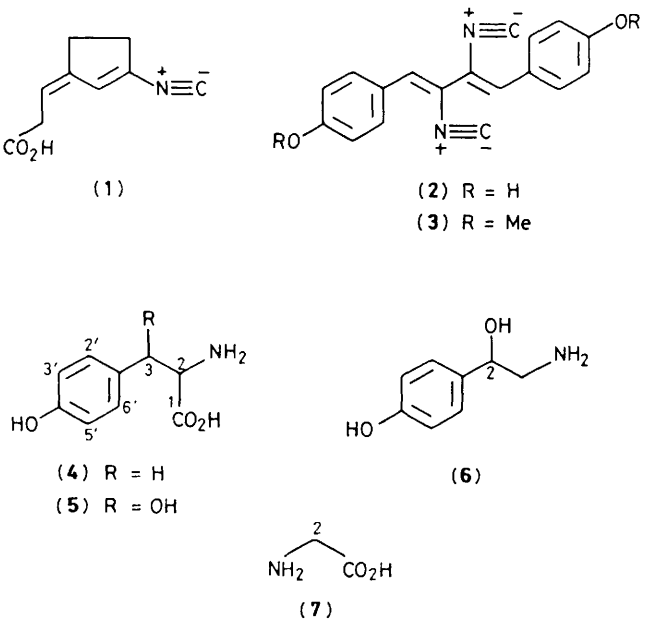
L-[U-^{14}C]Tyrosine (1.15 μCi; 504 mCi mmol^{-1}) was found to be a very efficient precursor for tuberin (**8**) in *S. amakusaensis* (ATCC 23876) (14—51% incorporation).† L-[1-^{14}C]Tyrosine (10 μCi; 56 mCi mmol^{-1}) acted as a very poor tuberin precursor (0.007%) which shows that the carboxy-group of (**4**) is lost during biosynthesis just as it is in the biosynthesis of (**1**)[1] and (**2**).[2] A check on the utilization of tyrosine as an intact C_8 unit, in experiments with L-[U-^{14}C,3′,5′-$^3H_2$]- and L-[U-^{14}C, 2′,6′-$^3H_2$]-tyrosine [as (**4**)] (similar weights and activities to other tyrosine samples above), revealed surprisingly that partial degradation of the tyrosine had occurred; the decrease in tritium relative to ^{14}C was 17—45% (in calculations, here and below, associated with mixed isotopic labelling of tyrosine, allowance was made for the loss of one ninth of the ^{14}C-activity arising from the loss of the carboxy carbon). That tritium was not being lost by exchange *ortho* to the phenolic hydroxy-group in the [3′,5′-$^3H_2$]tyrosine during biosynthesis was confirmed in an experiment with [U-^{14}C,3′,5′-$^2H_2$]tyrosine [as (**4**)] (84% $^2H_2$ species, 10 mg; 0.8 μCi). The tuberin obtained was shown, by mass spectrometry, to contain a significant amount of dideuteriated species but ⩽1% of monodeuteriated species.

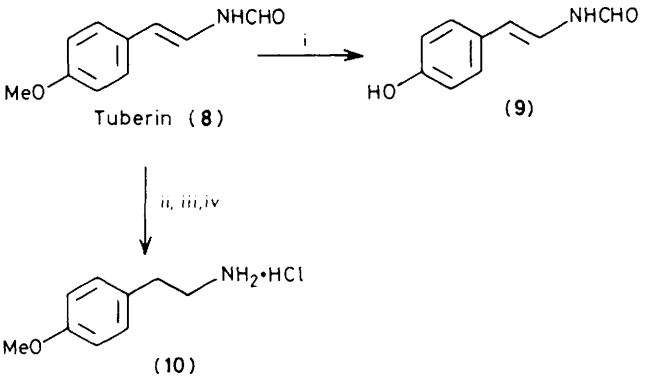
The specific incorporation‡ of ^{14}C-label into (**8**) was higher (10.9%) than that of deuterium (7.5%) but the specific incorporation measured on the degradation product (**10**) was very similar (7.8%) to the specific incorporation of deuterium into (**8**). It follows that the tyrosine fed had undergone partial degradation, and 15% of the ^{14}C-label from (**4**) was located in the *N*-formyl group of (**8**); alternative degradation of (**8**) to (**9**) was without loss of radioactivity (degradations were carried out on 2 samples of tuberin obtained from tyrosine in different experiments) which showed that no labelling of the *O*-methyl group of tuberin (**8**) by tyrosine had occurred.

[*Me*-^{14}C]Methionine (18.3 μCi; 56 mCi mmol^{-1}) served as an efficient precursor for tuberin (**8**) (21% incorporation). Essentially all the radioactivity was lost on conversion of the tuberin into (**9**). Thus the methyl group of methionine is a source of the *O*-methyl group in (**8**) but not of the *N*-formyl group. Neither [^{14}C]formate (3.2 μCi; 59 mCi mmol^{-1}) (0.02% incorporation) nor [2-^{14}C]acetic acid (5 μCi; 59 mCi mmol^{-1}) (0.01%) or [U-^{14}C]pyruvic acid (4 μCi; 16.6 mCi mmol^{-1}) (0.02%) were significantly incorporated into (**8**). Similar results have been obtained for (**2**).[2]

We considered that octopamine (**6**) or 3-hydroxytyrosine (**5**) might be involved as intermediates in tuberin biosynthesis. [2-^{3}H]Octopamine (2 mg; 25 μCi) was not significantly incorporated (0.05%) but DL-[2-^{14}C,3′,5′-$^2H_2$]-*threo*-3-hydroxytyrosine [as (**5**)] (synthesis following ref. 5) (11 mg; 3.2 μCi) served as a satisfactory precursor albeit at a lower level than tyrosine (1.0%). However, the tuberin (**8**) was devoid of deuterium (0.9% enrichment required for intact incorporation) and 60% of the ^{14}C-label was lost on degradation to (**10**). Thus (**5**) serves only as a C_1 source labelling the *N*-formyl group and, probably, the *O*-methyl group. It is known that glycine can serve as a C_1 source through methylene tetrahydrofolate[6] and has been noted to do so recently in the biosynthesis of another *Streptomyces* metabolite, naphthyridinomycin.[7] It is suggested, and supported by other results below, that labelled 3-hydroxytyrosine (**5**) is degraded by a reverse-aldol reaction to give [2-^{14}C]glycine [as (**7**)] which labels the C_1 units in (**8**). Since (**5**) is stable in aqueous solution under the conditions of the feeding experiment the putative reverse-aldol reaction is likely to be enzyme-mediated and the sequence (**4**) → (**5**) → (**7**) may account for the partial degradation observed on incorporation of tyrosine (**4**) into tuberin (**8**).§ However, the failure of [U-^{14}C]tyrosine, unlike labelled (**5**) and (**7**), to label the *O*-methyl group of (**8**) argues against this.

[2-^{14}C]Glycine (10 μCi; 49.5 mCi mmol^{-1}) was found to be a satisfactory precursor for tuberin (**8**) (1.6—3.2% incorpora-





Reagents: i, BBr_3; ii, H_2/Pd–C;[10] iii, 1 M aq. NaOH;[10] iv, HCl.[10]

† % Incorporation is: 100 × [total radioactivity in (**8**) isolated]/[total radioactivity in precursor].

‡ Specific incorporation a used here for ^{14}C and ^{2}H is: 100 × {amount of label per mmol of (**8**) [or (**10**)]}/{amount of label per mmol of precursor}.

§ It should be noted that the nitrogen atom and C-2 of tyrosine serve, as does alternatively glycine, as the source for a C_1–N unit in thiamine biosynthesis (refs. 8,9), but here the remainder of the tyrosine skeleton is converted into *p*-hydroxybenzyl alcohol (ref. 9) without intervention of the reverse-aldol product, *p*-hydroxyphenylacetaldehyde.

tion). The results of degradation to (**9**) and (**10**) showed that 56% of the activity was in the *N*-formyl group and 38% in the *O*-methyl group of tuberin (**8**), a very similar distribution to that deduced for the [2-^{14}C]-3-hydroxytyrosine. We conclude that C-2 of glycine is specifically the source for the C_1-units in tuberin probably *via* methylenetetrahydrofolate. It is possible that glycine may provide the *N*-formyl group of (**8**) instead by way of glyoxylic acid, its transamination product, but the close similarity in the labelling levels of the C_1 units in (**8**) argues against this.

Glycine can also serve as a precursor for (**3**).[4] Work is now in hand to define closely the origins of the isonitrile (**3**), the detailed biosynthetic pathways to (**8**) and (**3**), and the relationship of these pathways to one another.

We thank the University of Leeds for a Scholarship (to J. M.) and for financial support from its Research Fund.

Received, 10th June 1983; Com. 768

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