The Incorporation of C_1 Units in the Biosynthesis of Tuberin and Xanthocillin

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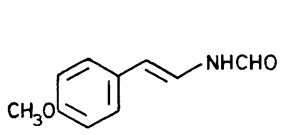
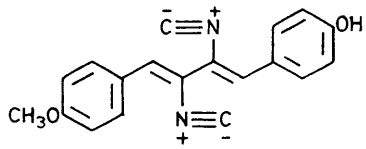
By a combination, particularly, of ^2H and ^{13}C labelling it is shown that glycine is incorporated into the *N*-formyl group of tuberin (**1**) with stereospecific loss of the 2-*pro-S* proton whereas both C-2 protons are retained in the *O*-methyl group, but there is also non-stereospecific loss of both protons in the formation of both C_1 groups; evidence is presented that the isonitrile carbon atoms in the xanthocillin (**2**) do not derive *via* the C_1-tetrahydrofolate pool.

Tuberin (**1**), a metabolite of *Streptomyces amakusaensis*,[1] is a simple, convenient metabolite in which to study C_1 metabolism, because it contains two C_1 units at different levels of oxidation. Study of the biosynthesis of tuberin also has potential bearing on the biosynthesis of naturally occurring isonitriles exemplified by xanthocillin (**2**). We report here on aspects of both tuberin and xanthocillin biosynthesis.

Previous results have shown that [2-^{14}C]glycine, but not [^{14}C]formate, labelled the two C_1 units in tuberin (**1**),[2] and to a similar extent. One concludes that the glycine is utilized by an orthodox pathway finally involving the important coenzyme, tetrahydrofolic acid.[3] We were interested to observe the fate of the two enantiotopic protons on C-2 of glycine in this widely occurring metabolic process,[4] by studying the formation of tuberin (**1**) in *S. amakusaensis*. We found in two experiments with a mixture of [2-^{14}C]- and [2-$^2H_2$]-glycine that the specific incorporation of ^{14}C was higher than that of deuterium, which was also somewhat variable (^{14}C: 7.4 and 6.8%; ^{2}H: 4.3 and 1.7%), *i.e.* deuterium appears to be lost from C-2 during biosynthesis, but this could be attributable to a deuterium isotope effect in which ^{14}C-labelled precursor (containing protium) was incorporated at a higher level than ^{2}H-labelled precursor. This aspect was examined by testing as precursor,



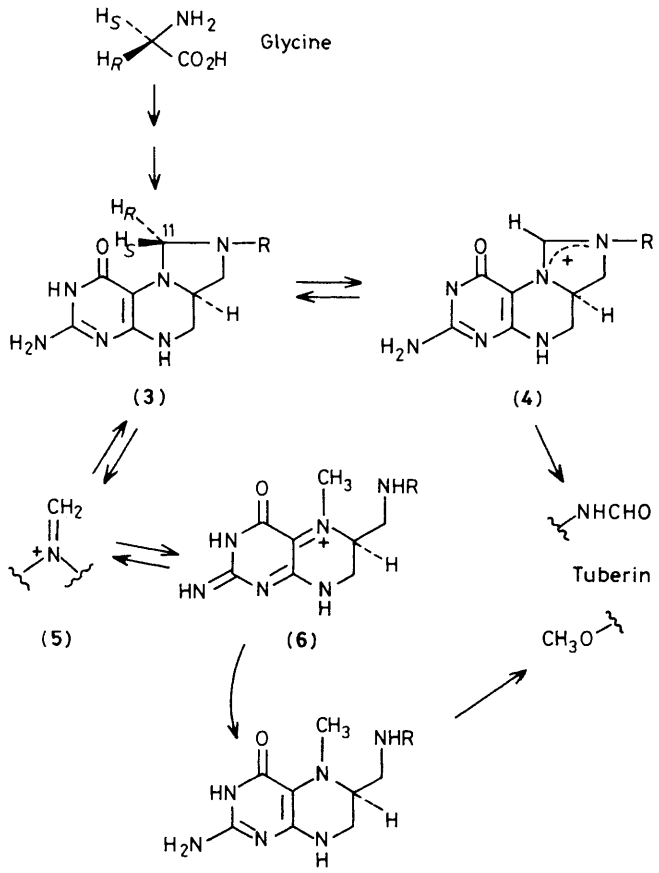
(**1**) Tuberin



(**2**)

glycine which contained both ^{2}H- and ^{13}C-labels in the same molecule.

[2-^{13}C, 2-$^2H_2$]Glycine (90% $^2H_2$; ^{13}C n.m.r.: quintet, *J* 22 Hz) gave tuberin (**1**), the ^{13}C n.m.r. spectrum of which showed: (a) substantially enhanced natural abundance singlets for the *N*-formyl and *O*-methyl groups, and these positions only; (b) a triplet associated with each of these signals shifted upfield by 0.16 and 0.27 p.p.m.,[5] respectively, and with $J(^{13}C–^2H)$ 30 and 22 Hz, *i.e.* for each group there is a ^{13}C species with a single deuterium atom attached; (c) a quintet for the *O*-methyl group (upfield shift 0.54 p.p.m., *J* 22 Hz), *i.e.* ^{13}C species with two deuterium atoms attached. Thus deuterium is being lost during biosynthesis. (2*R*)- and (2*S*)-[2-^{2}H]Glycine[6] mixed with [2-^{14}C]glycine each gave labelled tuberin (**1**) (specific enrichment of ^{14}C in each case 2.5%). The ^{2}H n.m.r. spectrum of the tuberin derived from

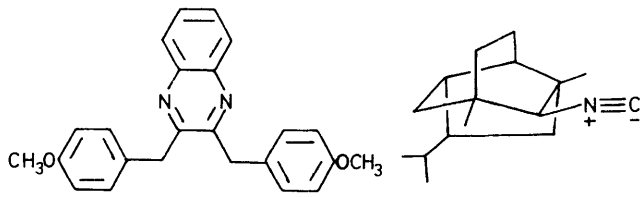


Scheme 1

the latter precursor showed a signal for the *O*-methyl group only, whereas the former precursor showed ^{2}H n.m.r. signals for both the *N*-formyl and *O*-methyl groups (correlation was obtained with the ^{1}H n.m.r. spectrum of tuberin). It follows from all these results that the *N*-formyl group does not arise *via* an isonitrile function (deuterium retention) as it does in another case.[7] One must conclude that biosynthesis is *via* normal tetrahydrofolate intermediates as for the *O*-methyl group (Scheme 1). The results also show: (a) that the conversion of C-2 of glycine into the *N*-formyl group of (**1**) involves stereospecific proton loss and this is the 2-*pro-S* proton, *i.e.* the conversion of methylenetetrahydrofolate (**3**) into methenyltetrahydrofolate (**4**) involves stereospecific removal of the glycine 2-*pro-S* proton; (b) that there is also partial non-stereospecific removal of both of the protons on C-2 of glycine. This latter finding may be accounted for tentatively by the equilibration during biosynthesis of (**5**) and (**6**) which has been advanced as a tetrahydrofolate intermediate in a pig-liver system by other workers.[8] The variable incorporation of ^{2}H compared to ^{14}C, noted above, is also consistent with an equilibration mechanism.

Our finding that the *in vivo* transformation of glycine *via* (**3**) and (**4**) into the *N*-formyl group of (**1**) is with stereospecific removal of the 2-*pro-S* proton ties in nicely with recent important results[9] which show that the 11-*pro-R* proton in (**3**) is removed during enzymatic conversion into (**4**). Thus, overall in the conversion of C-2 of glycine into C-11 of (**3**), the 2-*pro-S* proton in the amino-acid becomes the 11-*pro-R* proton in (**3**).

Initial results suggested that glycine was the source of the xanthocillin isonitrile carbon atoms, but rigorous experimentation using *Dichotomyces cejpii*[10] establishes that glycine is not a source of these carbon atoms in xanthocillin monomethyl ether (**2**). Thus [2-^{13}C, 2-^{14}C]glycine (6.6 μCi; 10.0 μCi mmol^{-1}) gave (**2**) which was found by ^{13}C n.m.r. spectroscopy to be labelled only on the *O*-methyl group (specific incorporation: 4.9% ^{14}C, 5.1% ^{13}C). The hydroxymethyl group in serine is a better source of C_1 units *via* tetrahydrofolate than is glycine.[3,11] L-[3-^{14}C]Serine was well incorporated into (**2**) (1.6% incorporation). Degradation[12] to (**7**), however, was without loss of radioactivity. Thus C-3 of serine is not a source of the isonitrile carbon atoms. A similar, negative result was obtained with [^{14}C]formic acid (22.65 μCi; 56 mCi mmol^{-1}; 5.0% incorporation into the xanthocillin) in agreement with the results of others.[13] [*Me*-^{13}C]Methionine labelled only the *O*-methyl group as expected (^{13}C n.m.r.; 6.5% specific incorporation). It is clear from these results, particularly the result with labelled glycine, that the C_1-tetrahydrofolate pool is labelled by the precursors fed. Since the isonitrile carbon atoms in (**2**) are not labelled, this C_1 pool is not the source of these carbon atoms. What this source is, remains a mystery. The biosynthesis of (**8**) produced by the sponge, *Hymeniacidon* sp., seems to be quite different,[7] and it is possible in this case that the isonitrile unit originates from cyanide ion.



(7) (8)

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