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# BIOSYNTHESIS OF TUBERIN AND XANTHOCILLIN FROM TYROSINE

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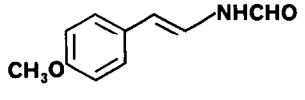
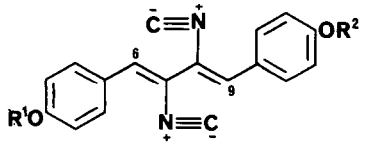
Summary: (2S)-Tyrosine (5) is incorporated stereospecifically into tuberin (1) with retention of the 3-pro-S proton and incomplete retention of the 2-S proton; a parallel pathway to the xanthocillin (4) is apparent with stereospecific retention of the 3-pro-R proton of tyrosine; results of experiments with threo- and erythro-3-hydroxytyrosine in relation to tuberin biosynthesis are reported.

The structures of tuberin (1) and xanthocillin (2) are at least superficially similar, and this similarity is sustained in a biosynthesis of both from tyrosine (5) with loss in each case of the carboxy-group of the amino-acid precursor.[1,2] Of particular interest in the biosynthesis of these structurally similar metabolites, which differ notably in double-bond geometry, is the stereochemistry/mechanism of the introduction of this unsaturation.

Neither tyramine (7) nor octopamine (8) are implicated in the biosynthesis of tuberin (1) in Streptomyces amakusaensis and racemic threo-3-hydroxytyrosine [as (9)] was only utilized after degradation; C-2 of this precursor served as a source for the C-1 units in (1).[2] This degradation plausibly occurs via a retro-aldol type reaction[3] to give 4-hydroxybenzaldehyde and glycine. In support, a cell free preparation of S. amakusaensis was obtained which converted threo-3-hydroxytyrosine into 4-hydroxybenzaldehyde, the conversion being approximately 45%, as appropriate for racemic material in an enzyme-catalysed reaction; racemic erythro-3-hydroxytyrosine[4] was inert in a parallel experiment. In accord with the above results, racemic [3',5'-$^{2}H_2$, 2-$^{14}C$]-erythro-3-hydroxytyrosine [as (9)] and [3,5-$^{3}H$]-4-hydroxybenzaldehyde (916 μCi; 22.3 mCi mmol) failed to label tuberin in intact cultures of S. amakusaensis. One concludes therefore that there is a normal, specific enzyme-catalysed degradation of one of the enantiomers of threo-3-hydroxytyrosine




(1)




(2) $R^1 = R^2 = H$
(3) $R^1 = R^2 = Me$
(4) $R^1 = Me$, $R^2 = H$

to give 4-hydroxybenzaldehyde in S. amakusaensis but this pathway does not lead to tuberin (1), except via glycine and the $C_1$ pool.

The results for tuberin (1) point away from C-3 hydroxylation of tyrosine, or its derivatives, being involved in the introduction of the double bond during the biosynthesis of this metabolite. A similar conclusion may be made for the biosynthesis of xanthocillin dimethyl ether (3)[5] in cultures of Aspergillus clavatus since neither (RS)-[2-$^3$H]-octopamine [as (8)] (20 μCi; 1.66 mCi mmol$^{-1}$) nor [2-$^{14}$C]tyramine [as (7)] (5 μCi; 56 mCi mmol$^{-1}$) served as significant precursors for this metabolite in contrast to labelled tyrosine which served as a good precursor.

As a probe into the mechanism of double-bond introduction in the biosynthesis of tuberin (1), tyrosine samples were prepared chirally deuteriated at C-3[6] and at C-2 (deuteriated by adaptation of a published procedure[7] and subsequent enzymic resolution[6]). The results of feeding experiments are shown in the Table. From experiment 2 it is clear that the (2S)-isomer (5) of tyrosine, and not the (2R)-isomer, is the precursor for tuberin [specific incorporation of tritium in the (2S)-precursor twice that for deuterium in the (2RS)-precursor] and the results of experiments 3 and 4 are in accord with this conclusion. From the results of experiments 1 and 2, it is clear that the E-double bond in tuberin (1) (J 14.5 Hz for the olefinic protons) is introduced by stereospecific removal of the 3-pro-R proton in tyrosine. Retention of deuterium at C-2 of (2S)-tyrosine (experiment 4) establishes that double-bond introduction must be associated with loss of the carboxy-group in tyrosine (for loss of the carboxy-group to have occurred after the introduction of the double bond all the deuterium at C-2 in the precursor would have to have been lost; some deuterium at C-2 is lost which is attributed simply to a competing transamination reaction). The mechanism then is, formally at least, an antiperiplanar elimination of hydrogen and carbon dioxide as observed for the conversion of, e.g., (10) into (11) in porphyrin biosynthesis.[8] The intermediate in which elimination occurs in tuberin biosynthesis may well be (6) and experiments to test this are in hand. This metabolism of tyrosine provides a nice contrast with the introduction of a double bond in the formation of dehydro-amino-acid moieties in several secondary metabolites. Where examined, loss of two atoms of hydrogen occurs here, and in a syn stereochemical sense.[9]

Experimental difficulties were experienced in the study of the biosynthesis of xanthocillin dimethyl ether (3) in A. clavatus cultures and we turned to the use of cultures of Dichotomomyces cejpii which has proved much more satisfactory. D. cejpii produces xanthocillin monomethyl ether (4).[10] Samples of (2RS,3R)- and (2RS,3S)-[3-$^2$H]-tyrosine were tested as precursors for (4). The metabolite was labelled by the former precursor (5.6% specific incorporation by mass spectral analysis with a single $^2$H n.m.r. signal at δ 8.45 assigned, on the basis of $^1$H n.m.r., to the hydrogen atoms on C-6 and C-9) but not by the latter. As in the case of tuberin biosynthesis above hydrogen is therefore removed stereospecifically from C-3 of tyrosine (5) but in the formation of (4) it is the 3-pro-S rather than the 3-pro-R proton which is removed in the course of

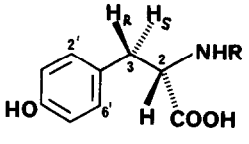
Table: Incorporation of labelled tyrosine samples into tuberin (1)

| Expt. | Tyrosine precursor | Specific incorporation (%)[a] | |
|---|---|---|---|
| | | $^{2}H$ | $^{3}H$ |
| 1 | (2R S,3R)-[3-$^{2}H$] + (2S)-[2',6'-$^{3}H_2$] | b | 26.1 |
| 2 | (2R S,3S)-[3-$^{2}H$] + " " | 12.6 | 25.1 |
| 3 | (2R)-[2-$^{2}H$] + " " | b | 14.7 |
| 4 | (2S)-[2-$^{2}H$] + " " | 1.4 | 10.2 |

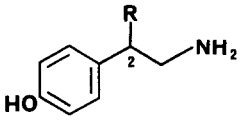
Specific incorporations of deuterium were calculated from mass spectral data. The specificity of the labelling was established from $^{2}H$ n.m.r. spectra where the signals, which were clearly observed, showed a close correlation with those in the $^{1}H$ n.m.r. spectrum of tuberin.

[a]Specific incorporation: 100 x (amount of label per mmol of product)/amount of label per mmol of precursor).

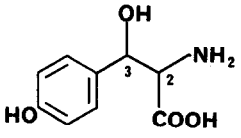
[b]Undetectable in $^{2}H$ n.m.r. spectrum and mass spectral enhancement of $M^{+}$ + 1 not significant.



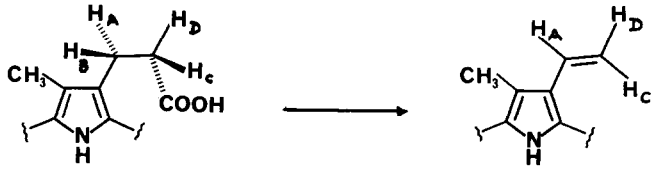

(5) R = H
(6) R = CHO




(7) R = H
(8) R = OH




(9)




(10) (11)

double bond formation. [The double bonds in (2) are proved by X-ray analysis to be as shown[11]]. This accords with the different geometries of the double bonds in the two metabolites and suggests that in the conversion of tyrosine into the xanthocillin (4) a similar antiperiplanar loss of hydrogen and the carboxy-group occurs.

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