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INCORPORATION OF PHENYLPROPANES INTO XYLERYTHRIN-TYPE PIGMENTS IN *PENIOPHORA SANGUINEA**

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Key Word Index—*Peniophora sanguinea*; Thelephoraceae; biosynthesis; fungus pigments; xylerythrin.

Abstract—Culture experiments with the fungus *Peniophora sanguinea* showed that the xylerythrin-type pigments of this organism are bio-synthesized via a pulvinic acid intermediate.

INTRODUCTION

Xylerythrin (**1**) [1] and the other known pigments from *Peniophora sanguinea*—5-*O*-methyl-xylerythrin (**2**) [2], peniophorin (**3**) [3], peniophorinin (**4**) [4], peniosanguin (**5**) [5, 6], and its Me ether (**6**) [5, 6]—are trimers derived from 3 monomer phenylpropane precursors. We tried to establish to what type of phenylpropane dimer intermediate the third phenylpropane unit was attached: (i) a grevillin [7] or a grevillin precursor, (ii) a derivative of polyporic acid [8], (iii) a derivative of pulvinic acid [9]. We also had to consider the possibility, that the results of the labelling experiments might only be caused by (iv) turn-over incorporation.

Particular consideration was given to the last point, since a long labelling period (5 weeks before extraction) [10] was necessary. At the end of this period and under optimum conditions maximum pigment production was about 3 μg per flask. Since the amount was so small, experiments with ^{13}C-labelled precursors were not feasible. Isolation of ^{14}C-labelled products by preparative-TLC or column chromatography also failed for this reason. Furthermore, the isolation of the main pigment, xylerythrin (**1**), by co-crystallisation with synthetic non-labelled xylerythrin gave erroneous results, as all the xylerythrin-type pigments have a tendency for mutual co-crystallisation with other pigments of this group. A degradation of such a 'crude' product would not give consistent results. An indirect method was therefore chosen, based on tyrosine incorporation, on the reasonable assumption that the oxidative step from phenylalanine (or analogous phenylpropanes) to tyrosine (or analogues) is not reversible.

RESULTS AND DISCUSSION

The decision as to which of the 4 discussed possibilities occurred was based on the fact that tyrosine could only be a precursor of those incorporated phenylpropanes which have a *p*-OH-substituted aromatic ring. Thus, xylerythrin (**1**) and its Me ether (**2**) have *one* such ring while peniophorin (**3**) and peniophorinin (**4**) have *two* [6]. For these two groups of pigments (with *one* or *two* *p*-OH-substituted rings) the respective incorporation of tyrosine caused by the 3 biosynthesis hypotheses (i to iii) is shown in Scheme 1. The relative results of all 4 possibilities are summarized in Table 1. As can be seen, during formation of the xylerythrin-skeleton one carboxy-group is lost. Therefore, one expects different labelling depending on whether the tyrosine side-chain is labelled-[1-^{14}C] or -[2-^{14}C].

In the first experiments *P. sanguinea* was grown on nutrient medium with the following precursors labelled-[1-^{14}C]: *trans*-cinnamic acid as control, since it was established that cinnamic acid is neither a precursor for the polyporic-type pigment volucrisporin [11] nor for the pulvinic-type pigments from *Pseudocyphellaria crocata* [12]. L-Phenylalanine as an absolute incorporation control. L-Tyrosine for realising a mutual incorporation of the C-1-carbon into those pigments with only *one* *p*-OH-substituted ring.

The result (Table 2) was an equivalent incorporation for all the pigments, for L-phenylalanine-[1-^{14}C] as well as for L-tyrosine-[1-^{14}C]. No incorporation could be detected for *trans*-cinnamic acid-[1-^{14}C].

Table 1. Expected results of incorporation into xylerythrin-type pigments after addition of tyrosine-[1-^{14}C] or tyrosine-[2-^{14}C] respectively*

	Xylerythin (1)	5-*O*-Methyl-xylerythrin (2)	Peniophorin (3)	Peniophorinin (4)
(i) Grevillin hypotheses	0/1	0/1	1/2	1/2
(ii) Polyporic hypothesis	0/1	0/1	1/2	1/2
(iii)				
(a) 1st pulvinic hypothesis	0.5/1	0.5/1	1.5/2	1.5/2
(b) 2nd pulvinic hypothesis	1/1	1/1	1/2	1/2
(iv) Turn-over results	$\frac{1}{n}/\frac{1}{m}$	$\frac{1}{n}/\frac{1}{m}$	$\frac{1}{n}/\frac{1}{m}$	$\frac{1}{n}/\frac{1}{m}$

* Numerical values presuming 100% end product labelling by the precursor, if incorporated by direct means. 1st value: tyrosine-[1-^{14}C]; 2nd value: tyrosine-[2-^{14}C].

* Part 5 of 'Studies on pigment-producing wood-fungi'; for Part 4 see Massow, F. v. and Tevini, M. (1975) (*Z. Pilzkunde* **41**, 99.

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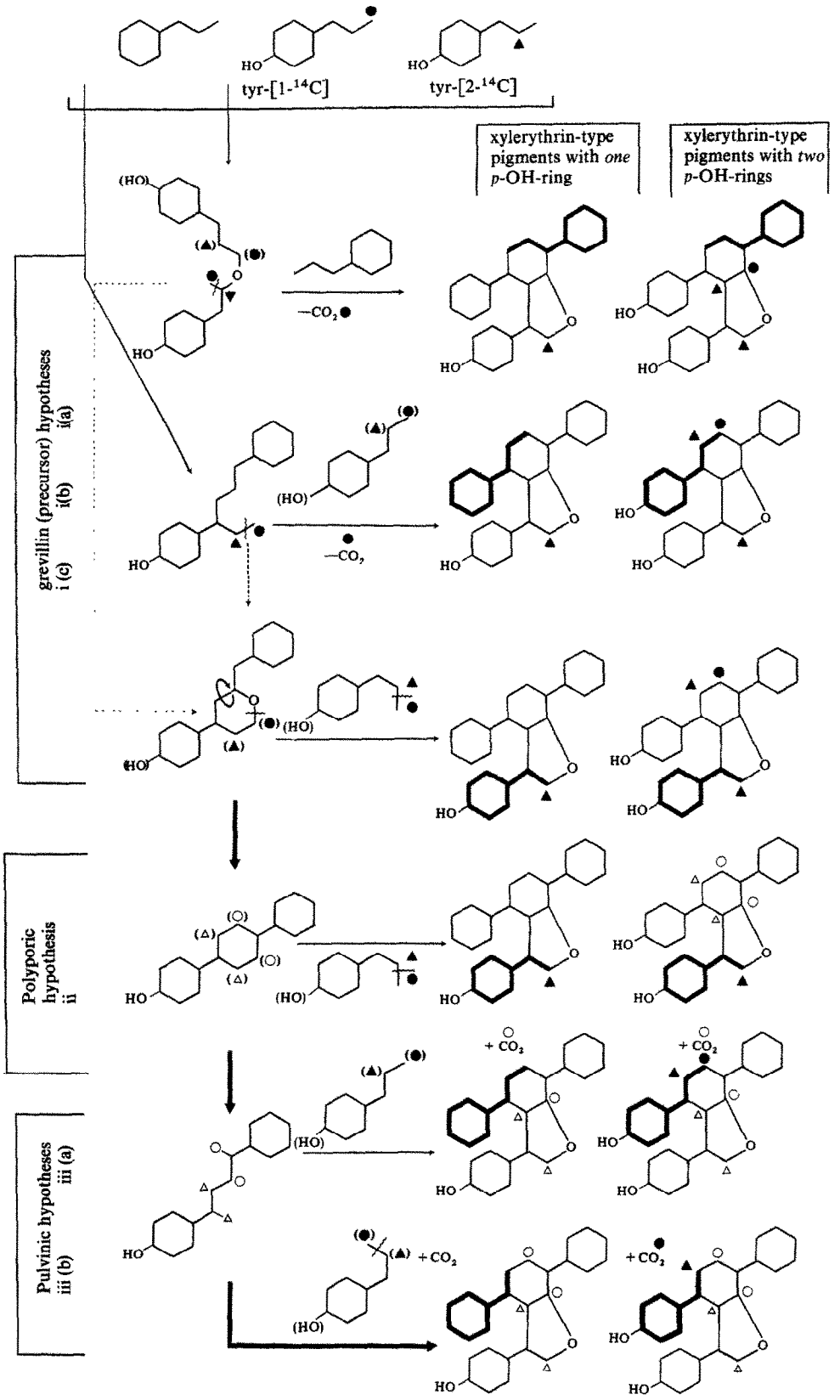


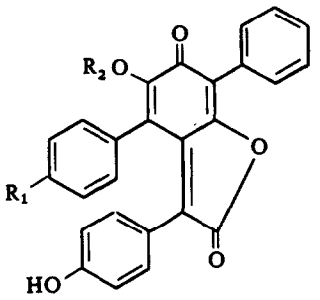
Scheme 1. Incorporation principle of tyrosine-[*1*-^{14}C] or -[*2*-^{14}C] respectively into the xylerythrin-type pigments with *one* or *two* *p*-OH-substituted ring(s) by the discussed biosynthetic pathway hypotheses. ● ▲ = full (100%) incorporation from the labelled carbon of the precursor(s). ○ △ = half (50% diluted) incorporation from the labelled carbon of the precursor(s).

Table 2. Relative incorporation rates of the known* xylerythrin-type pigments obtained from the experiments with L-phenylalanine-[1-^{14}C], L-tyrosine-[1-^{14}C] and D,L-tyrosine-[2-^{14}C]

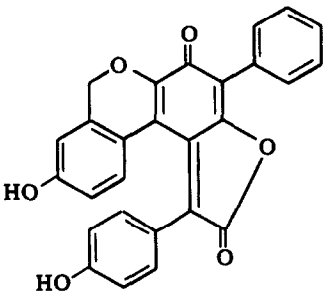
	Xylerythrin (1)			5-O-Methyl-xylerythrin (2)			Peniophorin (3)			Peniophorinin (4)		
	A	B	C	A	B	C	A	B	C	A	B	C
L-phenylalanine-[1-^{14}C]	2.04	4.64	1	2.10	6.18	1.31	1.83	4.82	1.01	1.59	4.82	1.04
○L-tyrosine-[1-^{14}C]	0.44	1	1	0.34	1	1	0.38	1	1	0.33	1	1
△D,L-tyrosine-[2-^{14}C]												
(a)†	1.01	2.3	1	0.96	2.68	1.17	—‡			1.73	4.55	1.98
(b)†	1.22	2.77	1	1.08	2.74	0.99	—‡			2.04	5.37	1.94
mean	1.12	2.54	1	1.02	2.71	1.08	—‡			1.89	4.96	1.96

A: Relative incorporation rate.
B: A-values normalized to the respective result for L-tyrosine-[1-^{14}C].
C: B-values normalized to the actual xylerythrin labelling.
*A hitherto unknown pigment (TLC polar; see u2 [19]) was isolated from 100 non-labelled cultures (0.8 mg) and it was shown (after HCl-treatment), that its chromophor is identical to xylerythrin. This u2 showed a C-value ratio of 1:1.13 tyr-[1-^{14}C]: tyr-[2-^{14}C].
† In (a) 12.5 μmol precursor, in (b) 25 μmol, but in both cases a total of 10 μCi. The nearly identical labelling of the product pigments in both cases indicates that the 2-fold higher concn of precursor in (b) is compensated by the 2-fold smaller sp. act.. Thus, in the concn range used precursor uptake increases linearly with concn, and essentially equivalent amounts of labelled precursor are taken up by the cells in the two cases. Similar results have also appeared in the literature [12].
‡ After addition of D,L-tyrosine-[2-^{14}C] this pigment was not detected. This may be a consequence of the different mycelium growth after addition of the L- or the D,L-form (see text).

The next step in the analysis required cultures with D,L-tyrosine [2-^{14}C]. Tyrosine turn-over should lead to non-diversified labelled pigments. However, its incorporation according to one of the pulvinic-hypotheses (iii(a) or iii(b)) should give labelling ratios of 1.33 : 2 or 2 : 1 for the pigments with *two* *p*-OH-substituted rings compared with those for the pigments with only *one* *p*-OH-group. A 2:1 ratio was found (Table 2), even when all the incorporation rates were higher than those of the experiments with L-tyrosine-[1-^{14}C]. The reason might be that



1: $R_1 = R_2 = H$
2: $R_1 = H/R_2 = Me$
3: $R_1 = OH/R_2 = H$



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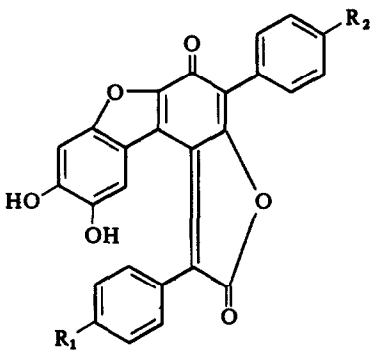

5: $R_1 = H/R_2 = OH$ or *vice versa*
6: $R_1 = H/R_2 = OMe$ or *vice versa*

these D,L-cultures grew slower than those with the L-form. Analogous stimulations are known from investigations on other plant pigments [13, 14].

The incorporation experiments are consistent with the second pulvinic-hypothesis (iii(b); Scheme 1 = thick arrows). A mathematical control, done by the χ^2-test [15], showed that neither the grevillin- nor the polyporic- nor the turn-over-hypothesis can be correlated with the results in Table 2 (probability 1 in 1 000 000). The first pulvinic-hypothesis explains one out of 100 cases. Only the second pulvinic-hypothesis gives a (normal) explanation (for about 90% of all the possible result combinations). Furthermore, a biological control was carried out (see [16]) which confirmed the above conclusions.

EXPERIMENTAL

Organism. Peniophora sanguinea (Fr.) Bres., strain Karlsruhe.

Cultures. 50 ml nutrient medium [17] in 250 ml flasks at room temp. in daylight. 12 weeks after inoculation 50 ml fresh medium and precursor (10 μCi) were added (L-phenylalanine-[1-^{14}C] or L-tyrosine-[1-^{14}C] or *trans*-cinnamic acid-[1-^{14}C] = 50 μmol; D,L-tyrosine-[2-^{14}C] = (a) 12.5 μmol or (b) 25 μmol). 5 weeks after precursor addition cultures were harvested by extracting [17] the pigments. To measure the amount of each pigment produced [18] and its actual radioactivity, TLC [19] and autoradiography [18] was carried out. As the pigments had not been isolated (see Introduction) the radioactive purity was checked by comparing the actual 'relative incorporation rates' from different TLC-systems. The maximum difference found was *ca* 12%. This is within the standard deviation of this complex system ($\sigma = \pm 13\%$). The 'relative incorporation rate' was calculated by dividing the actual radioactivity by the amount of substance. The results were expressed as units relative to the respective reference standard. To obtain the reference standard at the 5-week-stage the medium of some flasks (containing L-phenylalanine-[1-^{14}C] as precursor) was replaced by 50 ml fresh precursor-free medium. Then, after a second 5-week-period, these reference cultures were also harvested by extraction.

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