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BIOSYNTHESIS OF THE XYLERYTHRIN-TYPE PIGMENTS IN *PENIOPHORA SANGUINEA**

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

Abstract—The hypothesis that biosynthesis of the xylerythrin-type pigments occurs via a pulvinic acid intermediate was confirmed by controlled biological experiments.

INTRODUCTION

Previous labelling studies using side-chain ^{14}C-labelled phenylpropanes [1] indicated that the xylerythrin-type pigments are bio-synthesized via a pulvinic intermediate. To confirm this hypothesis *Peniophora sanguinea* was grown with (i) possible turn-over products, and (ii) pulvinic acid derivatives.

RESULTS AND DISCUSSION

(i) In view of the 5-week period necessary for labelling during the reported biosynthesis experiments [1] we added L-phenylalanine-[ring-^{14}C] or possible turn-over metabolites—acetate-[1-^{14}C], benzoic acid-[1-^{14}C] and phenylacetic acid-[1-^{14}C]. The autoradiograms of an analytical TLC, done by a '4-dimensional procedure', showed no labelling of the xylerythrin-type pigments for acetate or for the two acids, while phenylalanine-[ring-^{14}C] was well incorporated. However, phenylalanine-[ring-^{14}C] gave only weak labelling of the 2,4-dihydroxy-6-alkyl-benzoic acid derivatives, e.g. peniolactol [2], produced by the fungus. Acetate was intensively incorporated into this group of substances, which is predictable, since these substances are thought to originate from the acetate/malonate-pool. Similarly effective labelling has already been detected when L-phenylalanine-[1-^{14}C], L-tyrosine-[1-^{14}C] or D,L-tyrosine-[2-^{14}C] are added [1]. On the contrary, neither of the aromatic acids gave any incorporation result. This different manner of incorporation into the pigments or the 2,4-dihydroxy-6-alkyl-benzoic acid derivatives showed that the turn-over of the phenylpropane amino acids starts with fission of the phenylpropane unit into a C_2- and a C_6C_1-unit (e.g. acetate and benzoic acid, which will be further degraded to

Table 1. Incorporation of precursors into xylerythrin-type pigments *in vitro* produced by *Peniophora sanguinea*

	Xylerythrin (3)			5-*o*-Methyl-xylerythrin (4)			Peniophorin (5)			Peniophorinin (6)		
	A	B	C	A	B	C	A	B	C	A	B	C
4-Hydroxy-pulvinic acid-[^{14}C]	1.12	2.15	1.0	1.06	2.04	1.0	1.14	2.19	1.0	1.32	2.54	1.0
Pulvinic acid-[^{14}C]	0.96	1.63	0.76	0.77	1.31	0.64	1.12	1.90	0.87	1.06	1.80	0.71
LiPhen-[1-^{14}C]*	2.04	1.02	0.47	2.10	1.05	0.52	1.83	0.94	0.43	1.90	0.95	0.37
D-Tyr-[2-^{14}C]*	1.12	1.12	0.52	1.02	1.02	0.50	—	—	—†	1.88	0.95 (×2)	0.37 (×2)
L-Tyr-[1-^{14}C]*	0.44	0.44	0.21	0.34	0.34	0.17	0.38	0.38	0.17	0.33	0.33	0.13

A: Relative incorporation rates [1] found.

B: A-values corrected by calculation including the actual concentration‡, specific radioactivity, and the number of molecules which would be incorporated without loss of the labelled carbon.

C: B-values normalized to the respective result of the best precursor.

* For data see [1].

† This pigment has not been detected in any culture with D,L-tyr-[2-^{14}C] (for possible reason see [1]).

‡ In the range used we showed the precursor concentration to have a linear influence on the pigment labelling [1].

* 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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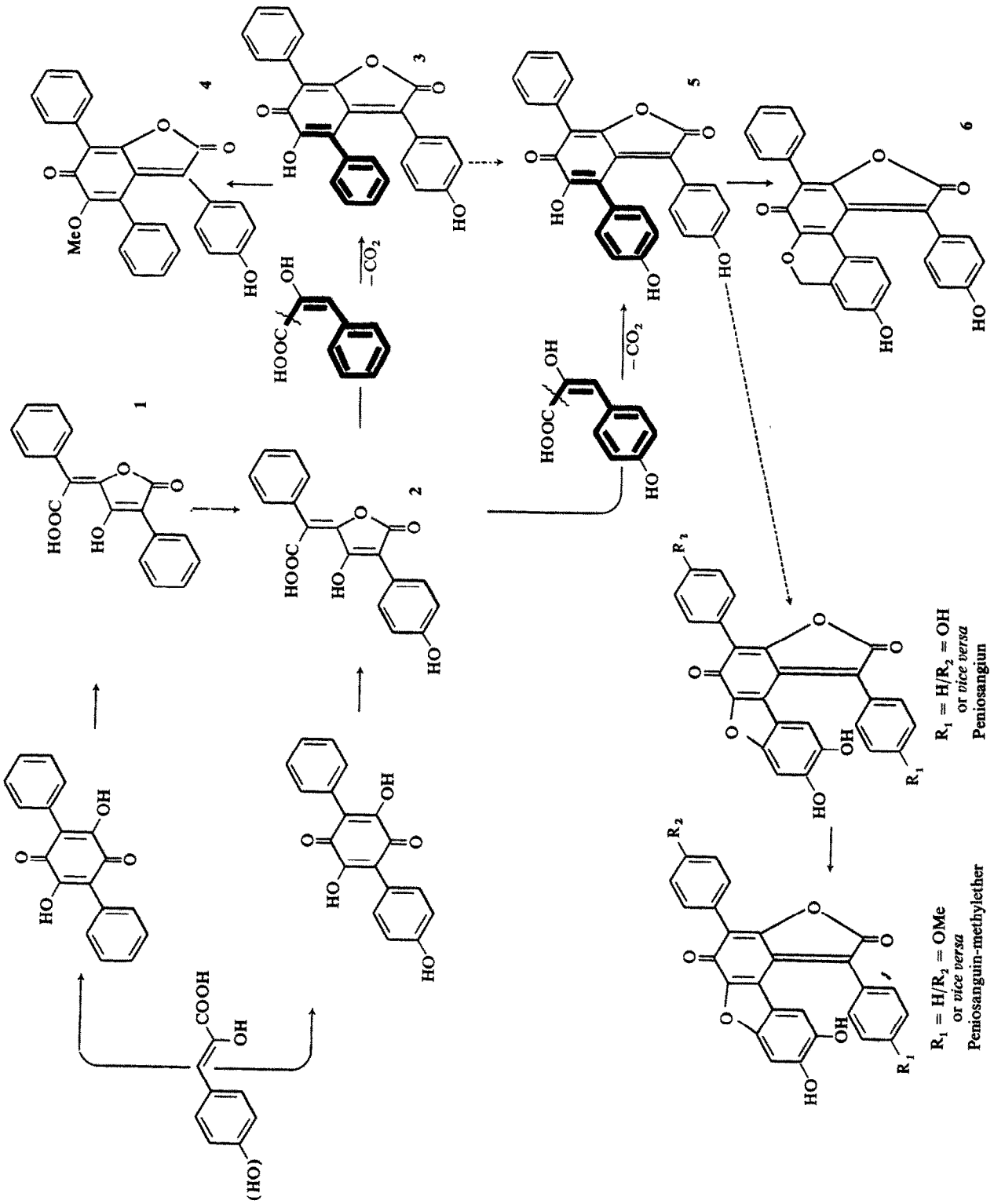

Scheme 1. Biosynthesis of the known xylerythrin-type pigments produced by the fungus *Peniophora sanguinea*.

acetate). This fission must be assumed as the initial step, because acetate as well as the side-chain [1-^{14}C] or -[2^{14}C] labelled amino acids lead to an intense incorporation into the probable products of the acetate/malonate-pool. However, the side-chain turn-over does not result in labelling of the pigments, consistent with previous conclusions.

(ii) Since previous work indicated that a pulvinic intermediate is included into the biosynthesis of the *Peniophora* pigments, we performed further control experiments using pulvinic acid-[^{14}C] (**1**) [3] and 4-hydroxy-pulvinic acid-[^{14}C] (**2**) [3]. Better incorporation results than those found for the phenylpropane amino acids [1] are expected. If the introduction of the *p*-OH group could take place at the dimer stage as well as via incorporation of monomer *p*-OH precursors, then both of the pulvinic acid labels should be incorporated and, in addition, the 4-hydroxy-pulvinic acid should be better incorporated than the unsubstituted one. The results (Table 1) are consistent with the above expectations. Furthermore, the pulvinic acid additions were followed by a 20-fold increase in pigment production.

Both groups of control experiments therefore confirmed the biosynthesis of the xylerythrin-type pigments via a pulvinic intermediate. The formation of the known pigments of this type obtained from cultures of *P. sanguinea* can be summarized as shown in Scheme 1. A similar way of quinone-ring formation by synchronous decarboxylation is known for naphthoquinones [4] and anthraquinones [5].

EXPERIMENTAL

Organism and culture conditions. As described earlier [1, 6].

Precursor concentrations. L-Phenylalanine-[ring-^{14}C] = 50 μmol (10 μCi); phenylacetic acid-[1-^{14}C] = 50 μmol (25.74 μCi); benzoic acid-[1-^{14}C] = 50 μmol (5.25 μCi); pulvinic acid-[^{14}C] [3] = 10 μmol (5.9 μCi); 4-hydroxy-pulvinic acid-[^{14}C] [3] = 10 μmol (5.18 μCi).

TLC (4-dimensional procedure). Starting position at the bottom left. 1st direction Me_2CO—cyclohexane (1:1), 2nd after a 90° turn to the left by system I described earlier [7]; then turn once more 90° to the left. With this plate orientation the main component (= group of 2,4-dihydroxy-6-alkyl-benzoic acid derivatives; see also [7]) appears in the middle of the right half. Immediately to its left a strip of the TLC-layer is scraped off. Thus divided into two parts (left without, right with the main component) the TLC-plate is developed and impregnated by Me_2CO–formamide (9:1). After a final turn of 180°, the part containing the main component is developed with system III [7] and the other part with system II [7]. Autoradiography of the TLC was done according to ref. [8].

2,4-Dihydroxy-6-alkyl-benzoic acid derivatives. The main component (= b [7]) of the culture extracts [6] consists of a group of closely related compounds (min 3). PMR: (90 MHz; $CDCl_3$; TMS) of the crude *product* δ 0.87 (3H, *t*), δ 1.25 (14H, *s*), δ 1.78 (2H, *pent*), δ 2.51 (2H, *t*), δ 6.31 (2H, *s*). *Analysis:* C, 69.78; H, 9.45. These data are closely related to those from penioactol [2], and they lead to a (mean) $C_{10/12}$-alkyl side chain.

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