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BIOSYNTHESIS OF ASPULVINONES. AROMATIC HYDROXYLATION META TO A PREEXISTING HYDROXYL GROUP

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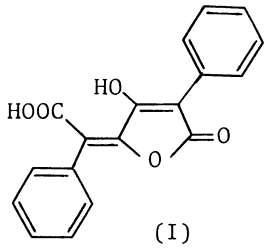
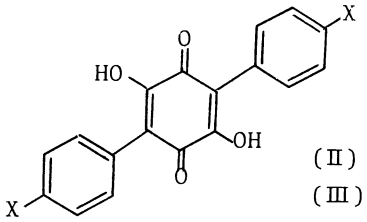
Shikimic acid, phenylalanine, and tyrosine are all incorporated into aspulvinones in *Aspergillus terreus*. The hydroxylation at C-4 involves the NIH shift, whereas C-2 is hydroxylated with loss of tritium from that position.

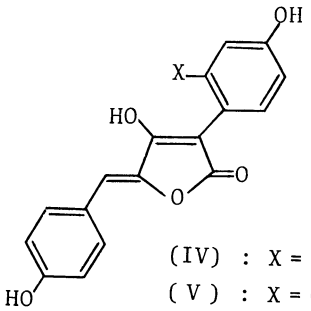
It has been shown that pulvinic acid (I) is biosynthesized from phenylalanine *via* polyporic acid (II).[1,2] By analogy aspulvinones, metabolites of *Aspergillus terreus*[3-7] are also assumed to be synthesized by way of the shikimate pathway, but aspulvinones are unique, because some of them have a resorcinol moiety which is characteristic of the polyketide pathway rather than the shikimate pathway. If they are derived from phenylalanine, two hydroxyl groups should be introduced to the aromatic ring in *meta* relation. Since such a process seems novel, we have studied the biosynthesis of these metabolites with a special attention to the *meta* hydroxylation.



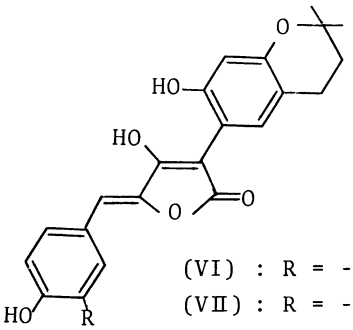
(I)



(II) : X = H
(III): X = OH



(IV) : X = H
(V) : X = OH



(VI) : R = $-CH_2-CH=C(CH_3)_2$
(VII) : R = $-CH_2-CH(O)-C(CH_3)_2$

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Shikimic acid, phenylalanine, tyrosine, and mevalonic acid were administered to a stationary culture of *A. terreus*, and aspulvinones were isolated after three weeks as described in the previous paper.[3] Since both phenylalanine and tyrosine were incorporated efficiently into aspulvinones (Table 1), feeding experiments with ^{3}H, ^{14}C-doubly labeled precursors were carried out. A mixture of L-phenylalanine-*ring*-4-*t* and L-phenylalanine-*carboxy*-^{14}C, a mixture of L-tyrosine-*ring*-2,6-t_2 and L-tyrosine-*U*-^{14}C, and a mixture of L-tyrosine-*ring*-3,5-t_2 and L-tyrosine-*U*-^{14}C were administered, and aspulvinone E (IV), G (V), D (VI), and F(VII) were isolated and methylated with diazomethane. The methyl ethers were purified to a constant $^{3}H/^{14}C$ ratio by thin layer and high-speed liquid chromatography. The $^{3}H/^{14}C$ ratios of the precursors and the derived products are given in Table 2. The retention of 72% tritium in IV formed from phenylalanine-*ring*-4-*t* shows that the NIH shift takes place in fairly high probability during the hydroxylation at C-4 of the aromatic ring. Based on this value the tritium retention in both VI and VII are consistent with that just one half of the tritium remaining in IV is lost by prenylation, indicating that the tritium at C-4 migrates to the neighboring positions as expected for the NIH shift.[9] On the other hand, the tritium retention during the formation of V, VI or VII from tyrosine-ring-2,6-t_2 is slightly larger than the value (75%) calculated for the loss of one fourth of the tritium, indicating that the hydroxylation at C-2 proceeds with almost complete loss of tritium from that position. The value of tritium retention in VI and VII derived from tyrosine-*ring*-3,5-t_2 (Exp. No. 7) was somewhat smaller than expected on the basis of that in IV and V, but the reason is not known.

Table 1. Incorporation of ^{14}C-labeled precursors into aspulvinones

Exp. No.	Precursor (μCi)	Product	Incorporation, %
1	Shikimic-*G*-^{14}C acid (1.4)	IV	0.5
		VI	5.9
		VII	5.8
2	L-Phenylalanine-*carboxy*-^{14}C (5.0)	IV	0.4
		VI	1.8
		VII	1.8
3	L-Tyrosine-*U*-^{14}C (2.5)	IV	13.0
		VI	6.7
		VII	5.0
4	Mevalonic-2-^{14}C acid (1.2)	IV	0.0
		VI	4.5
		VII	3.0

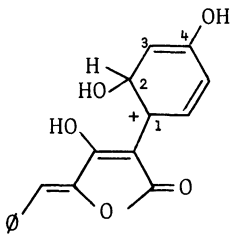
Table 2. Incorporation of doubly labeled precursors into aspulvinones

Exp. No.	Precursor	$^3H/^{14}C$ Ratio of precursor	Product	$^3H/^{14}C$ Ratio[a] of product	Retention of ^3H, %
5	L-Phenylalanine-*ring-4-t*; *carboxy*-^{14}C	10.82	IV	7.80	72.1 (100)[b]
			V	7.97	73.7 (102)
			VI	3.90	36.0 (50)
			VII	3.95	36.5 (51)
6	L-Tyrosine-*ring-2,6-*t_2; *U*-^{14}C	6.11	IV	5.95	97.4 (100)
			V	4.71	77.1 (79)
			VI	4.80	78.6 (81)
			VII	4.64	75.9 (78)
7	L-Tyrosine-*ring-3,5-*t_2; *U*-^{14}C	12.18	IV	10.94	89.8 (100)
			V	10.90	89.5 (100)
			VI	4.32	35.5 (40)
			VII	4.54	37.3 (42)

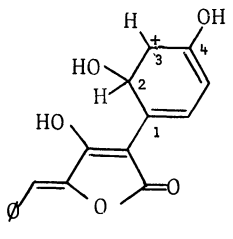
a) The correction for the loss of one carbon atom during aspulvinone formation was made by multiplying the observed ratio by three-quarters[8] and seventeen-eighteenths for the experiments using phenylalanine-*carboxy*-^{14}C and tyrosine-*U*-^{14}C, respectively.

b) The figures in parentheses indicate the percentage of retention based on that of IV.

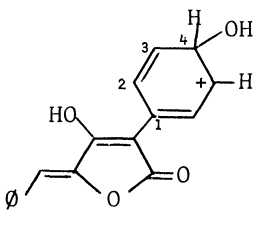
It would be reasonable to assume that V is formed by the hydroxylation of IV in which the conjugated enol system attached to C-1 has an effect to inhibit the NIH shift by neutralizing the positive charge generated when the epoxide ring of the 1,2- or 2,3-arene oxide, possible intermediates in the hydroxylation, is opened as shown in A and B. In contrast, C-4 must be hydroxylated at the C_3— C_6 stage, probably at phenylalanine . If there were an alternate route where phenylalanine is converted into IV not by way of tyrosine but, for example, *via* I, the extent of the NIH shift should not be so large as observed, because a similar effect of the conjugated enol system in I would also be valid for the hydroxylation at C-4 as exemplified by formula C.



(A)



(B)



(C)

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8. This is based on the assumption that aspulvinones are formed *via* atromentin (III) in which the four carbons originated from the carboxyl and α-carbon of phenylalanine or tyrosine are indistinguishable. Cf. reference 1 and R. J. Wikholm and H. W. Moore, J. Amer. Chem. Soc., 94, 6152 (1972).
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