

## Biosynthesis of Pulvinone Derivatives in *Aspergillus terreus*. Enzymatic Prenylation of Dihydroxypulvinone

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**Summary** The crude ammonium sulphate fraction of a cell-free extract from *Aspergillus terreus* catalysed the transfer of the C<sub>5</sub> units from 3,3-dimethylallyl pyrophosphate (**2**) to both of the aryl rings of dihydroxypulvinone (**1**), giving a possible precursor of the metabolites (**4**) and (**5**).

PREVIOUSLY, we reported the isolation and the structure of dihydroxypulvinone (1) and its prenyl derivatives such as (4) and (5).<sup>1,2</sup> We now report the enzymatic synthesis of a

new prenyl derivative (3) from (1) and 3,3-dimethylallyl pyrophosphate (2) in a cell-free system prepared from *A. terreus*.

Washed mycelia of 6 day-old culture of this fungus were ground in a mortar with sea sand and four volumes of buffer, pH 7.0, containing 10 mM each of Tris-HCl, MgCl<sub>2</sub>, and EtSH. The slurry was centrifuged at 28,000 × g for 30 min, and the enzyme was isolated from the supernatant liquid by fractionation with ammonium sulphate. The most active fraction was precipitated between 33 and 55% saturation. The standard incubation mixture contained, in a final volume of 1 ml, 10 μmol of MgCl<sub>2</sub>, 10 μmol of KF, 0.3 μmol of [<sup>14</sup>C]-(1) (0.05 Ci/mol), 1 μmol of (2), 25 μmol of Tris-HCl buffer, pH 7.0, and ca. 0.5 mg enzyme protein. The radioactive (1) was prepared by *in vivo* synthesis from L-[<sup>14</sup>C]tyrosine administered to the culture of the same fungi. The incubation was carried out at 37 °C for 2 h, and the radioactive material was extracted with ether, and was subjected to t.l.c. (silica, Et<sub>2</sub>O : n-C<sub>6</sub>H<sub>12</sub> : MeCO<sub>2</sub>H = 40 : 8 : 1).

The radio-t.l.c. showed a major radioactivity peak at *R<sub>f</sub>* 0.59 and a minor peak at *R<sub>f</sub>* 0.70 in which reference compounds (1), (4), and (5) had peaks at *R<sub>f</sub>* 0.31, 0.65, and 0.75, respectively. The ether-extractable material {60%, based on [<sup>14</sup>C]-(1)} showed little radioactivity when either the enzyme or (2) was omitted from the complete incubation mixture. Experiments using [<sup>3</sup>H]-(2) (5.1 Ci/mol) and non-labelled (1) as substrates also gave similar radio-t.l.c. The mass spectrum of the major product showed *m/e* 432 (*M*<sup>+</sup>) corresponding to C<sub>27</sub>H<sub>28</sub>O<sub>6</sub>, and the fragmentation pattern was similar to that of (4) or (5). Furthermore, treatment of this compound with toluene-*p*-sulphonic acid at 120 °C gave (5). These results indicate that the prenylation occurred at the expected position on each of the two aryl rings of (1).

However, it is not clear whether the product has the structure (3) or the partially cyclized (6), the former seeming likely from the observed t.l.c. mobility which is much smaller than that of (4). The product from a large incubation was purified by liquid-chromatography, and the n.m.r. spectrum showed the methyl groups as a single peak at δ 1.80 (12H). From this it was concluded that the product of the enzymatic reaction between (1) and (2) was the prenylated derivative (3), a possible precursor for (4) and (5).

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<sup>1</sup> N. Ojima, S. Takenaka, and S. Seto, *Phytochemistry*, 1973, **12**, 2527.

<sup>2</sup> N. Ojima, S. Takenaka, and S. Seto, *Phytochemistry*, 1975, **14**, 573.

