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

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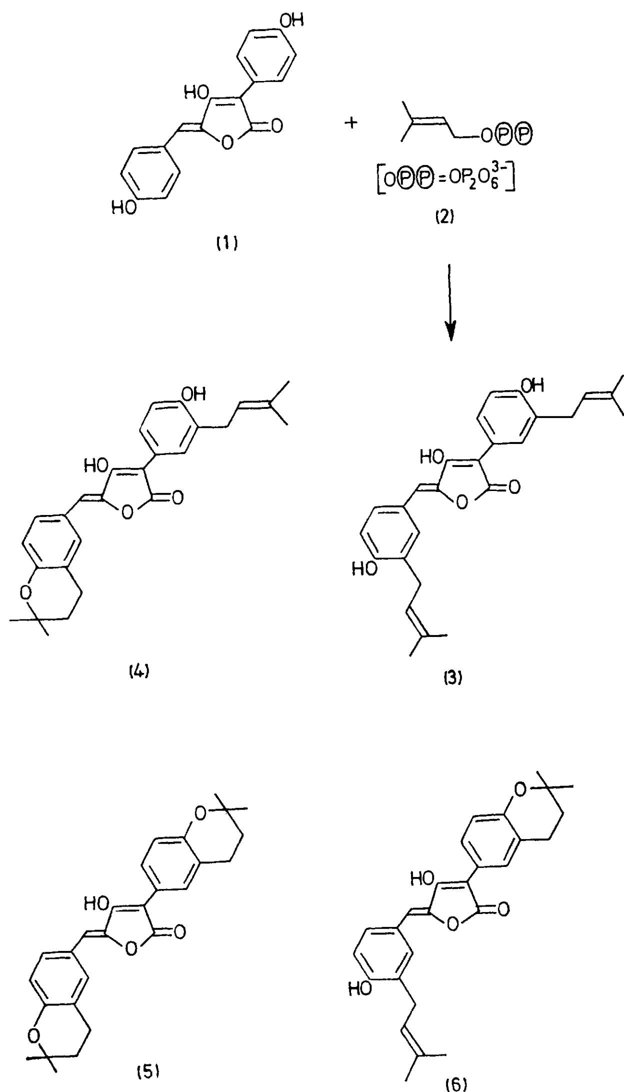
Summary The crude ammonium sulphate fraction of a cell-free extract from *Aspergillus terreus* catalysed the transfer of the C_5 units from 3,3-dimethylallyl pyrophosphate (**2**) to both of the aryl rings of dihydroxypulvinone (**1**), giving a possible precursor of the metabolites (**4**) and (**5**).

Previously, we reported the isolation and the structure of dihydroxypulvinone (**1**) and its prenyl derivatives such as (**4**) and (**5**).[1,2] 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, $MgCl_2$, 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 $MgCl_2$, 10 μmol of KF, 0·3 μmol of [^{14}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-[^{14}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, $Et_2O : n\text{-}C_6H_{12} : MeCO_2H = 40 : 8 : 1$).

The radio-t.l.c. showed a major radioactivity peak at R_f 0·59 and a minor peak at R_f 0·70 in which reference compounds (**1**), (**4**), and (**5**) had peaks at R_f 0·31, 0·65, and 0·75, respectively. The ether-extractable material {60%, based on [^{14}C]-(**1**)} showed little radioactivity when either the enzyme or (**2**) was omitted from the complete incubation mixture. Experiments using [^{3}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^+) corresponding to $C_{27}H_{28}O_5$, 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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[1] N. Ojima, S. Takenaka, and S. Seto, *Phytochemistry*, 1973, **12**, 2527.
[2] N. Ojima, S. Takenaka, and S. Seto, *Phytochemistry*, 1975, **14**, 573.