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FACILE ENZYMATIC SYNTHESIS OF LABELLED GERANYL PYROPHOSPHATE

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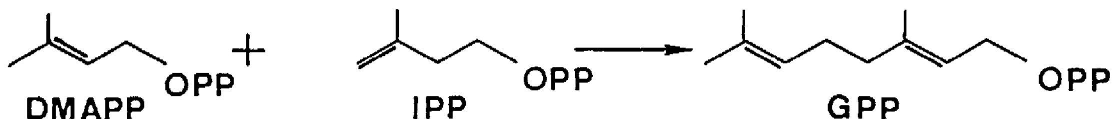
Summary: $[1-^{14}C]$ geranyl pyrophosphate was prepared from $[1-^{14}C]$ isopentenyl pyrophosphate and dimethylallyl pyrophosphate in 50 % yield, using a purified geranyl pyrophosphate synthase from *Lithospermum erythrorhizon* cell cultures. This procedure provides a simple route to different stereo- and regiospecifically labelled geranyl pyrophosphates.



Geranyl pyrophosphate (GPP) is a key intermediate in the biosynthesis of monoterpenes and other isoprenoid compounds, and isotope-labelled geranyl pyrophosphates are essential tools for the investigation of these pathways[1]. So far, labelled GPP had to be prepared by pyrophosphorylation of geraniol, which was usually labelled with ^{2}H or ^{3}H at position 1 by reduction of the corresponding aldehyde[2]. However, this procedure often causes some contamination with the cis-isomer and the 2,3-dihydro-compound[3]. An enzymatic preparation of GPP from dimethylallyl pyrophosphate (DMAPP) and isopentenyl pyrophosphate (IPP) would open an easier access to a range of stereo- and regiospecifically labelled geranyl pyrophosphates, since chemical and enzymatical methods are available for the preparation of specifically labelled IPP and DMAPP[3-5], and since the stereochemical course of the prenyltransferase reaction is well known[3]. However, an enzymatic synthesis of GPP has not been possible up to now, since all known prenyltransferases yielded farnesyl pyrophosphate (FPP) or longer prenyl pyrophosphates rather than GPP as final products[3]. Only recently, indications for the occurrence of specific GPP synthases have been found[1,6-8], and a crude cell-free extract from *Rosa dilecta* has been used for the enzymatic preparation of $[1-^{14}C]$geraniol[8].

We have partially purified a GPP synthase from cell cultures of *Lithospermum erythrorhizon* Sieb. et Zucc.[9]. The purified enzyme was free from phosphatase and DMAPP-IPP isomerase activities, and the sole product of the reaction was GPP. In the present study, this enzyme was used for a convenient preparation of $[1-^{14}C]$GPP, which could be obtained by a simple TLC

purification in 50 % yield.

The enzyme solution[9] was gel-filtrated over Sephadex G-25, which had been equilibrated with KP_i 0.005 M pH 6.75, 20µM PMSF, 0.1 mM EDTA. The incubation mixture contained in a final volume of 1.86 ml: [1-^{14}C] IPP (Amersham), 0.847 µmol (1.74 MBq); DMAPP, 2.5 µmol; $MgCl_2$, 4.5 µmol; KP_i pH 6.75, 8.75 µmol; and GPP synthase, 0.23 mg (1.75 nKat). After incubation for 60 min at 30°C, the reaction was terminated by the addition of 5.5 µmol EDTA and 25 µl conc. NH_4OH. The solution was concentrated by a gentle flow of N_2 and applied to a cellulose TLC plate (20 cm x 20 cm x 0.1 mm; Merck, W. Germany), which had been pre-developed in H_2O - conc. NH_4OH (3:1). The plate was developed with n-PrOH - conc. NH_4OH - H_2O (6 : 3 : 1). The area containing [^{14}C] GPP (Rf 0.78) was localized by radioscanning, scraped off and eluted in a column with 5 ml NH_4HCO_3 5 mM. Yield 0.874 MBq (50 %).

Radiochemical purity: $>$96 %, assayed in the TLC system described above. The spots corresponding to IPP and GPP (Rf 0.49 and 0.76, resp.) were scraped off, eluted with H_2O and assayed by scintillation counting; IPP contained $<$0.7 % of the total activity.

Contamination with FPP: 3.300 Bq of the product were hydrolized by incubation with 40 mg acid phosphatase (Sigma) at pH 4.7 at 37 ° for 3 h. After addition of 25µl NaOH 5 N and 0.1 mg each of farnesol and geraniol, the mixture was extracted with 2 x 1 ml petrol ether. The organic layer was concentrated by a stream of nitrogen, applied to a Whatman KC 18 F reversed phase TLC plate and developed in acetone - H_2O (7 : 3). Detection: I_2 and radioscanning. The spots corresponding to geraniol and farnesol (Rf 0.41 and 0.20, resp.) were scraped off, eluted with MeOH and assayed by scintillation counting; farnesol contained $<$ 0.7 % of the total activity.

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