

NOTE

ENZYMATIC SYNTHESIS OF LABELLED GERANIOL

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**Summary:**  $[1-^{14}\text{C}]$ Geraniol was conveniently prepared from  $[1-^{14}\text{C}]$ isopentenyl pyrophosphate and dimethylallyl pyrophosphate in 56 % yield.

**Key words:** geraniol; synthesis; prenyl transferase (EC 2.5.1.1); geranyl pyrophosphate synthase

Labelled geraniol is usually prepared by the reduction of geranial, introducing  $^2\text{H}$  or  $^3\text{H}$  at position 1 [1]. A disadvantage of this procedure is a possible contamination with the *cis*-isomer or the 2,3-dihydro compound [2]. An enzymatic preparation of  $[^{14}\text{C}]$ geraniol, using crude extracts from *Rosa* *dilecta* flowerheads, has been reported [3]. The procedure reported here is a refinement of that approach.

0.76  $\mu\text{mol}$   $[1-^{14}\text{C}]$ isopentenyl pyrophosphate (1.65 MBq; Amersham) were incubated with 2.25  $\mu\text{mol}$  dimethylallyl pyrophosphate, 4.5  $\mu\text{mol}$   $\text{MgCl}_2$ , 60  $\mu\text{mol}$   $\text{KPi}$  (pH 7.5) and 100 units of purified geranyl pyrophosphate synthase [4] in a total volume of 1620  $\mu\text{l}$ . After 110 min at 30°C, pH was adjusted to 4.8 with acetic acid, and 4 mg (120 units) of acid phosphatase (from sweet potato; Sigma) in 200  $\mu\text{l}$  sodium acetate buffer (0.7 M; pH 4.8) were added. The mixture was overlaid with 1 ml pentane and incubated for 60 min at 37°C. 3.5 ml

EtOH were added to prevent extraction of any isopentenol [5], and the aqueous phase was extracted five times with 4 ml pentane. Pentane phases were combined and filtered through cotton. Yield 0.93 kBq (56 %) [ $1-^{14}\text{C}$ ]geraniol. Acid hydrolysis [6] of the aqueous phase yields additional 0.39 kBq (23 %) [ $1-^{14}\text{C}$ ]geraniol; this procedure, however, may give rise to the formation of isomers. HPLC analysis [6] of the product of enzymatic hydrolysis showed >97 % radiochemical purity; neither nerol nor farnesol or isopentenol could be detected.

Since the stereochemical course of the prenyl transferase reaction is known, this procedure opens a simple route to different stereo- and regiospecifically labelled geraniol derivatives. Alternative sources for purified geranyl pyrophosphate synthase have been described [7, 8].

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References:

1. Croteau R. and Karp F. - *Arch. Biochem. Biophys.* **176**, 734 (1976)
2. Poulter C.D. and Rilling H.C. - *Biosynthesis of Isoprenoid Compounds*, Vol. I (J.W. Porter, L. Surgeon, eds.) J. Wiley & Sons, New York 1981; p. 161
3. Banthorpe D.V. and Branch S.A. - *J. Labelled Comp. Radiopharm.* **25**, 913 (1988)
4. Heide L. and Berger U. - *Arch. Biochem. Biophys.* **273**, 331 (1989)
5. Barnard G.F. - *Meth. Enzymol.* **110**, 155 (1985)
6. Heide L. - *FEBS Letters* **237**, 159 (1988)
7. Croteau R. and Purkett P.T. - *Arch. Biochem. Biophys.* **271**, 524 (1989)
8. Suga T. and Endo T. - *Phytochemistry* **30**, 1757 (1991)