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FORMATION OF A NONALLYLIC HOMONERYL PYROPHOSPHATE BY FARNESYL PYROPHOSPHATE SYNTHETASE

Akio SAITO, Kyozo OGURA, and Shuichi SETO

Chemical Research Institute of Non-Aqueous Solutions,
Tohoku University, Katahira 2-1-1, Sendai 980

The reaction of 4-methylpent-4-enyl pyrophosphate (1) with dimethylallyl pyrophosphate (2) catalyzed by farnesyl pyrophosphate synthetase (prenyltransferase) results in an exclusive *cis* condensation to give a nonallylic homoneryl pyrophosphate (3).

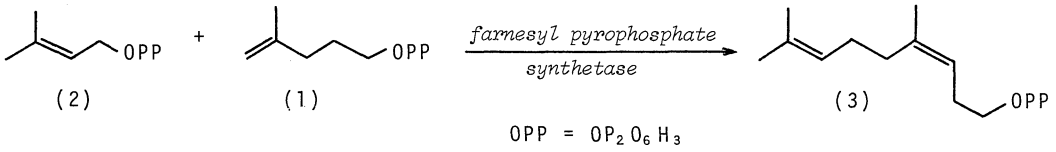
We reported previously that 4-methylpent-4-enyl pyrophosphate (1) acted as an artificial substrate for farnesyl pyrophosphate synthetase, reacting with geranyl pyrophosphate to give a nonallylic *cis*-homofarnesyl pyrophosphate.
The enzymatic reaction of this substrate (1) with dimethylallyl pyrophosphate (2) remained unknown because the reactivity was extremely low in contrast to that in the reaction between 1 and geranyl pyrophosphate. However, the unusual behavior of 1 found in the reaction with geranyl pyrophosphate has stimulated us to study also the reaction between 1 and 2. The interest is focused on whether 1 reacts with 2 to give a homolog of the nonallylic type for which the enzymatic reaction is forced to stop at this intermediary step, and whether the stereochemistry of the condensation is also exclusively *cis*.

In order to obtain the product in an amount sufficient for the structural investigation, the following incubation was carried out. The incubation mixture contained, in a final volume of 5 ml, 125 μmol of Tris-HCl buffer (pH 7.5), 25 μmol of $MgCl_2$, 500 nmol of [^{3}H]-labeled 2 (5 x 10^5 dpm), 500 nmol of 1, and 0.5 mg of farnesyl pyrophosphate synthetase. The enzyme was purified from pig liver essentially by the method of Holloway and Popják[2] as modified by us in that DEAE Sephadex chromatography was applied at the final step of purification.[3] The mixture was kept at 37°C for 5 hr and was then treated with alkaline phosphatase as usual. The radioactive materials were extracted with ether, and were subjected to radio-gaschromatography with 1 m OV 17, 1.5% at linear programmed temperature at a rate of 4°C/min from 100 to 200°C. A single radioactivity peak was observed at retention time of 1.25 relative to that for geraniol. Incubation was also carried out using non-radioactive 2, and the phosphatase hydrolysate was analyzed by GC-MS. The product showed peaks at m/e 168 (M^+, $C_{11}H_{20}O$), 150 (M-18), 125 (M-43), 81 (M-18-69), and 69 (C_5H_9) with relative intensities of 0.33, 0.29, 17.3, 12.4, and 100%, respectively. These results indicate that the product was homogeraniol or its *cis* isomer, homonerol. The yield was 0.3% based on 2.

For a further identification, authentic samples of homogeraniol and homonerol were chemically synthesized. Treatment of geranyl cyanide with boiling ethanolic KOH gave a 1:2 mixture of the *cis* and *trans* carboxylic acids, the methyl esters of which

were separated from each other by preparative gaschromatography. The retention volume of the *cis* isomer relative to that of *trans* isomer was 0.91 on a 1 m column of OV 17, 1.5% at 120°C. The *trans* ester shows signals for the methyl groups at δ 1.63 (6H), and 1.67 (3H), whereas the *cis* isomer shows signals for the methyls at δ 1.62 (3H), 1.68 (3H), and 1.75 (3H). The *cis* and *trans* esters were reduced to homonerol and homogeraniol, respectively. Although these two alcohols showed an identical retention volume on gaschromatography, the corresponding acetates were separable from each other, the retention volume of homoneryl acetate relative to that of homogeranyl acetate being 0.91 on 1 m PEG 20M, 20% at 140°C.[3]

Therefore, for the identification of the product of the enzymatic reaction, the alcohol obtained by the phosphatase treatment was acetylated, and the acetate was analyzed by GC-MS. Both the retention time and mass spectrum of the enzymatically derived acetate were identical with those of homoneryl acetate. Thus, it was evidenced that the enzymatic reaction of 1 with 2 resulted in an exclusive formation of homoneryl pyrophosphate (3) and that no further condensation of 1 with 3 occurred.



(2) (1) (3)

OPP = $OP_2O_6H_3$

Farnesyl pyrophosphate synthetase catalyzed both dimethylallyl-transferring reaction (the condensation of isopentenyl pyrophosphate with 2 to form geranyl pyrophosphate) and geranyl-transferring reaction (the condensation of isopentenyl pyrophosphate with geranyl pyrophosphate to form *trans,trans*-farnesyl pyrophosphate), the reactivity of the former relative to that of the latter being 0.63.[4] However, the reactivity of 1 with 2 was as low as 0.01 relative to that of 1 with geranyl pyrophosphate. This remarkable difference in the relative reactivity is compatible with our assumption that the enzyme has independent catalytic sites for the dimethylallyl-transferring and geranyl-transferring reactions.

The *cis* condensation of 1 can be explained by a model similar to that proposed previously for the formation of *cis*-homofarnesyl pyrophosphate.[1]

References and Notes

1) K. Ogura, A. Saito, and S. Seto, *J. Amer. Chem. Soc.*, 96, 4037 (1974).
2) P. W. Holloway and G. Popjāk, *Biochem. J.*, 104, 57 (1967).
3) Homonerol and homogeraniol were also synthesized, though in a very low yield, by the respective reduction of neryl cyanide and geranyl cyanide followed by the diazotization, and the corresponding acetates were identified with those obtained as described in the text.
4) T. Nishino, K. Ogura, and S Seto, *Biochim. Biophys. Acta*, 302, 33 (1973).

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