NON ENZYMIC FORMATION OF NEROLIDOL FROM FARNESYL PYROPHOSPHATE IN THE PRESENCE OF BIVALENT CATIONS

C. George-Nascimento, R. Pont-Lezica, and O. Cori
Laboratory of General Biochemistry
Faculty of Chemistry and Pharmacy,
University of Chile, Santiago, Chile

Received July 26, 1971

SUMMARY: 1-^{3}H Farnesyl pyrophosphate is hydrolyzed non enzymically at neutral or alkaline pH in the presence of Mg^{2+} or Mn^{2+}. The pattern of product formation differs for these two ions. In the presence of Mn^{2+}, nerolidol is practically the only reaction product, whereas in the presence of Mg^{2+} the hydrolysis products are nerolidol and farnesols. The effect of Mg^{2+} is pH dependent. The formation of nerolidol from mevalonic acid or other precursors of isoprenoids may thus be explained as a non enzymic reaction of farnesyl pyrophosphate.

INTRODUCTION: Nerolidyl pyrophosphate, a tertiary sesquiterpene ester has been postulated as an intermediate in the biosynthesis of squalene from farnesyl-pyrophosphate (FPP) on the basis that radioactive nerolidol has often been found to be formed from 2-^{14}C mevalonic acid or from other precursors (1) (2) (3). Nerolidyl pyrophosphate has not been isolated as such, and its biosynthetic role is still somewhat obscure (4).

We have observed that cell free preparations obtained from Pinus radiata seedlings (5) or from orange rind (6) form labelled nerolidol from various precursors, in spite of the precautions taken to avoid acid catalyzed hydrolysis and rearrangement of allylic pyrophosphates (2) (3). If the deproteinized aqueous phase from such an enzymic experiment was stored before treating it with E.coli alkaline phosphatase to release prenols from phosphorylated intermediates (5), we observed a marked increase in the amount of nerolidol that could be extracted with hexane. This led us to suspect non enzymic hydrolysis of FPP with allylic rearrangement even in the absence of acid (3). This communication presents data to support this view.

EXPERIMENTAL: All chemicals used were reagent grade. ATP was obtained from Sigma Chemical Co., St. Louis, Missouri, and isopentenyl pyrophosphate from Mann Research Laboratories, New York. Alkaline phosphomonoesterase from E.coli was obtained from Worthington Biochemical Corporation, Freehold, N.J. Potato apyrase was a gift of Prof. A. Traverso, University of Chile.

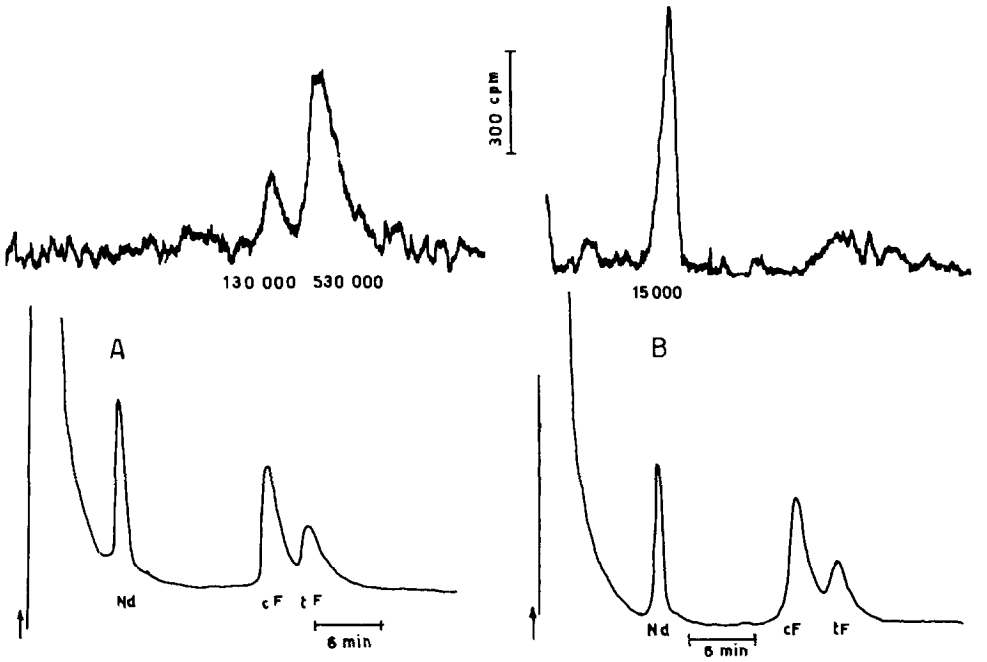
Nerolidol and farnesol were obtained from Aldrich Chemical Co., Milwaukee, Wisc. 2 cis, 6 trans and 2,6 di trans farnesol were separated from the commercial mixture by distillation in a Nester-Faust spinning band column (7). They were identified by NMR spectrometry (8).

1-^{3}H FPP synthesized chemically (3) was a generous gift from Prof. G. Popják, U.C.L.A. Hydrolysis of this compound with alkaline phosphomonoesterase plus potato apyrase (5) released about 20% of 2 cis, 6 trans farnesol and 80% of 2,6 di trans farnesol (Fig 1A).

Incubations of 2 ml samples in glass stoppered tubes were carried out for 2 hours at 37°. After cooling in ice, the reaction products were extracted with 2 ml of hexane containing 300 µg of each carrier prenol. Radioactivity was measured in an aliquot by conventional scintillation spectrometry (5). Reaction products were analyzed in another aliquot by gas chromatography using a thermal conductivity detector at 200° and a stainless steel column of 300 x 0.635 cm packed with alkali washed (8) Chromosorb W 60-80 mesh coated with 2% ethyleneglycol adipate. Helium flow: 50 ml/min.

The effluent gas was passed into a heated proportional counter (Biospan 4998, Nuclear Chicago). Radioactivity and carrier peaks were recorded by a double pen instrument. Radioactivity was evaluated by comparison with the peak areas obtained using 1-^{3}H geraniol (9).

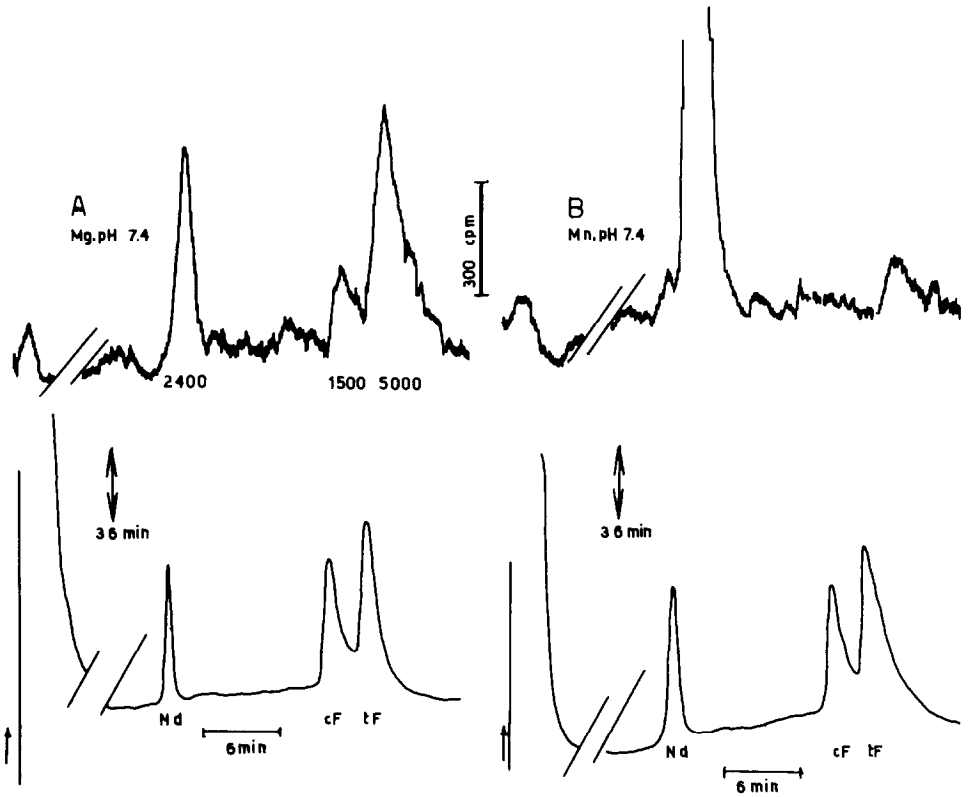
RESULTS AND CONCLUSIONS: Complete enzymic hydrolysis of 1-^{3}H FPP produced no detectable amounts of nerolidol (Fig 1A). This result excludes possible allylic rearrangements of farnesols on the column. On the other hand, incubation of FPP in the presence of heat inactivated enzymes from Pinus seedlings in the medium currently used in our biosynthetic experiments (fig 1B) produced almost exclusively nerolidol and only traces of farnesols. About 2% of the added FPP was hydrolyzed in two hours.



-FIGURE 1.- Gas chromatographic analysis of the products of enzymic and non enzymic hydrolysis of Farnesyl pyrophosphate.

670 000 cpm (0.15 µmoles) of 1-^{3}H FPP were incubated as follows:
A- 25 mM TRIS-HCl buffer pH 8.4;15 mM $MgCl_2$;0.05 mg of phosphatase from E.coli and 50 Units of potato apyrase.
B- 25 mM TRIS-HCl buffer pH 7.4;5.0 mM ATP; 10 mM mercaptoethanol;0.4 mM isopentenyl pyrophosphate; 2.5 mM $MgCl_2$;2.5 mM Mn Cl_2 and heat inactivated enzyme (5 min at 100°) from P. radiata seedlings (5) corresponding to 2 mg of native protein.
Gas chromatography was performed isothermically at 160°. The upper tracings indicate radioactivity and the lower indicate carrier mass. The arows indicate sample injection. Carrier peaks are standard nerolidol (Nd), 2 cis, 6 trans farnesol (cF) and 2,6 di trans farnesol (tF). Total radioactivity in cpm per tube is indicated under the radioactivity peaks.

If we used a crude extract from P. radiata seedlings (5) at pH 7.4 instead of the boiled enzyme, 10% of the radioactivity of FPP was released as hexane soluble products, 1.5% being detected as nerolidol and 8.5% as the sum of both farnesols. This shows that during enzymic hydrolysis of FPP, some nerolidol was formed. Under these experimental conditions, the Pinus acid phosphatase (5) is much slower than the E.coli enzyme, and there is sufficient unhydrolyzed FPP to undergo rearrangement.



-FIGURE 2.- Gas chromatographic analysis of the products of non enzymic hydrolysis of Farnesyl pyrophosphate in the presence of Mg^{2+} or Mn^{2+}.

670 000 cpm of 1-^{3}H FPP were incubated in 50 mM TRIS-HCl buffer pH 7.4 in the presence of either A-10 mM $MgCl_2$ or B- 10 mM $MnCl_2$.

Gas chromatography was performed with the following program: 20 min isothermically at 115°, 5 min of a linear rise to 155° and 40 or more min isothermically at 155°. The early part of the chromatogram has been omitted as indicated by the interruptions of the graph and by the double arrow at 36 min. Other indications as in Fig 1.

Fig 2 shows that non enzymic hydrolysis of FPP at pH 7.4 in the presence of Mg^{2+} ions occurred with rearrangement of about 25% of the hydrolysis products. On the other hand nerolidol was practically the only product in the presence of Mn^{2+}. In the absence of added metals or in the presence of Ca^{2+} there was a slight degree of hydrolysis, but no nerolidol could be detected. In the presence of equimolar concentrations of both cations, Mn^{2+} sets the pattern of products.

In comparable experiments at pH 8.4 there was no formation of nerolidol in the presence of Mg^{2+} and less than 1% of the radioactivity of FPP was found in both farnesols. Again, the presence of Mn^{2+} promoted rearrangement: about 1% of the counts added were found in nerolidol and none in the farnesols.

The results presented show qualitatively that acid catalysis (10) (11) (12) is not the only condition for the rearrangement and hydrolysis of allylic pyrophosphates. There is a definite pattern in the hydrolysis products, which suggests that Mn^{2+} somehow favours C-O fission with ensuing rearrangement, whereas both C-O and P-O fission may occur in the presence of Mg^{2+}. A similar effect of Mg^{2+} and Mn^{2+}, with marked differences in the pattern of product formation has been observed in the hydrolysis of phosphate esters of nerol and geraniol (13). The mechanism of these reactions is being explored.

It is worth stressing that Mg^{2+} or Mn^{2+} are required by several enzymes that participate in the formation of isoprenoids from mevalonic acid. These ions are always added in biosynthetic experiments (1) (2) (6) (12) (14). It is thus conceivable that if there is an adequate steady state concentration of FPP, these ions may be responsible for the formation of nerolidol. The occurrence of this non enzymic rearrangement should be carefully ruled out when assesing the significance of nerolidol (15) or its esters (3) (16) as well as of other tertiary prenols (6) (16) (17) in the biosynthesis of natural products.

ACKNOWLEDGEMENTS: This work was supported by research grants from the Comisión de Investigación Científica, Universidad de Chile, and from CONICYT, Chile. The use of facilities of the University of California and the participation of Prof. C. A. Bunton were possible through an interuniversity Exchange Program, funded by the Ford Foundation. NMR spectroscopy was performed by Mr. D. Hachey, University of California, Santa Bárbara. C. George-Nascimento held a fellowship from CONICYT; R. Pont-Lezica is an O.A.S. Postdoctoral Fellow.

REFERENCES:

1. Popják, G. and Cornforth, J.W., Advan. Enzymol. 22-281 (1960)
2. Goodman, De W.S. and Popják, G., J. Lipid Research 1-286 (1960)

3. Popják, G.; Edmond, J.; Clifford, K. and Williams, V., J. Biol. Chem. 244-1897 (1960)
4. Sofer, S.S. and Rilling, H.C., J. Lipid Research 10-183 (1969)
5. Beytía, E.; Valenzuela, P. and Cori, O., Arch. Biochem. Biophys. 129-346 (1969)
6. George-Nascimento, C. and Cori, O., Phytochemistry 10 (1971) in press.
7. Corey, E.J. and Achiwa, K., Tetrahedron Letters 3257 (1969)
8. Bates, R.B.; Gale, D.M. and Gruner, B.J., J. Org. Chem. 28-1086 (1963)
9. Van Aller, R.T. and Nes, W.R., Phytochemistry 7-85 (1968)
10. Valenzuela, P. and Cori, O., Tetrahedron Letters 3015 (1967)
11. Cramer, F. and Rittersdorf, W., Tetrahedron 23-3015 (1967)
12. Lynen, F.; Eggerer, H.; Henning, U. and Kessel, I., Angew. Chem. 70-738 (1958)
13. Bunton, C.A., and Cori, O., Unpublished observations.
14. Holloway, P.W. and Popják, G., Biochem. J. 104-57 (1967)
15. Ruzicka, L., Experientia 9-357 (1953)
16. Attaway, J.A., Pieringer, A.P. and Buslig, B.S., Phytochem. 6-25 (1967)
17. Potty, V.H., and Bruemmer, J.H., Phytochem. 9-1229 (1970)