SYNTHESIS OF 1-^{3}H-3, 3-DIMETHYLALLYL PYROPHOSPHATE.

E. Cardemil★ and O. Cori★.

Received on June 22, 1972.

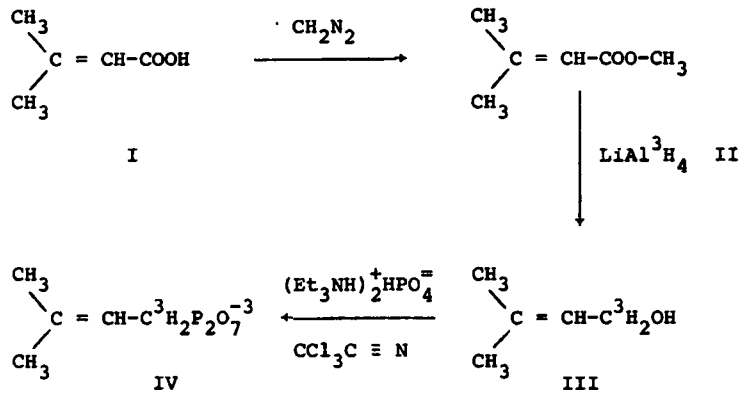
SUMMARY

3,3-Dimethylallyl pyrophosphate (DMAPP) labeled with ^{3}H on C-1 was prepared from the corresponding acid by esterification, reduction of the methyl ester with $LiAl^{3}H_{4}$ and pyrophosphorylation of the radioactive alcohol. The products were purified by ion-exchange chromatography, yielding a single radioactive compound. Phosphorus analysis and gas chromatography showed that the final product was DMAPP.

INTRODUCTION

In connection with our work on the biosynthesis of mono and sesquiterpenes in plant tissues (1,2) it appeared necessary to obtain ^{3}H labeled 3,3-dimethylallyl pyrophosphate. The methods described so far produce ^{14}C labeled compounds (3,4) and we wanted to study the biosynthesis of doubly labeled ten carbon compounds taking advantage of the commercial availability of 1-^{14}C-isopentenyl pyrophosphate. The method described in this communication is based on the reduction of the methyl ester of 3,3-dimethylacrylic acid with $LiAl^{3}H_{4}$ and subsequent pyrophosphorylation of the 1-^{3}H-3,3-dimethyl-allyl alcohol (5,6).

● Laboratory of General Biochemistry
Faculty of Chemistry and Pharmacy
University of Chile
Casilla 233, Santiago 1, Chile.



Similar procedures have been described for other prenols (7). The synthetic compound was biologically active.

EXPERIMENTAL

Methyl 3,3-dimethylacrylate (II)

Two hundred mmoles of 3,3-dimethylacrylic acid (I) (Chemical Procurement Laboratories, N.Y.) were treated for 30 min at room temperature with an ether solution of 33.4 mmoles of diazomethane obtained from N-methyl-N-nitroso-p-toluenesulfonamide (Diazald, Aldrich Chemical Co., Milwaukee, Wisc.). The crude ester was purified by distillation at 40 torr. The fraction distilling at 43° was collected. $n_D^{19} = 1.438$; $d_4^{18} = 0.938$ (reported values (8) $n_D^{20} = 1.432$; $d_4^{20} = 0.9337$). Gas chromatographic analysis of the ester on a 300 x 0.63 cm column of Chromosorb W 60-80 coated with 2% ethylene glycol adipate showed a single peak with a retention volume of 159 ml at 90°, as compared with 243 ml for dimethylallyl alcohol (kindly furnished by Dr. A. Hortmann, Dep. of Chemistry, Washington University, St. Louis).

1-^{3}H-3,3-Dimethylallyl alcohol (III)

A mixture of 19 mg $LiAlH_4$ and 1.9 mg (5 mCi) $LiAl^3H_4$ (New England Nuclear Co., Boston, Mass.) corresponding to a final specific radioactivity of 5 x 10^6 dpm/µgatom of H was suspended in 2 ml of anhydrous ether and refluxed for 60 min. A solution of 0.2 ml

(1.63 mmoles) of methyl 3,3-dimethylacrylate dissolved in 0.8 ml of dry ether was added dropwise over a period of 10 min at room temperature. A flocculent precipitate appeared. The reaction mixture was stirred for further 10 min and refluxed for 15 min. It was cooled to 0° and the reaction stopped by the addition of one ml of ethyl ether previously saturated with water and about 200 mg of solid ammonium chloride. The mixture was extracted with ether three times. The combined ether extracts were extracted twice with saturated $NaHCO_3$ solution, dried over $CaCl_2$ and concentrated to 5.5 ml. Gas chromatographic analysis (1) showed that 97% of the radioactivity emerged with carrier 3,3-dimethylallyl alcohol and 3% emerged in a region corresponding to the rearrangement product dimethylvinylcarbinol (Chemische Fabrik, Fluka AG., Switzerland). The yield of this step was 68%. The specific radioactivity could be calculated to be 2.6×10^6 cpm/μmole of alcohol.

1-^{3}H-3,3-Dimethylallyl pyrophosphate (IV)

The ethereal solution of 1-^{3}H-3,3-dimethylallyl alcohol containing 1.9×10^9 cpm, estimated by conventional liquid scintillation spectrometry with 26% efficiency, was concentrated to a final volume of 0.1 - 0.2 ml with a stream of dry nitrogen. Two ml of anhydrous ether were added and the operation was repeated twice. After the final evaporation 0.8 ml of Cl_3CCN was added with stirring and after 45 min a solution of 300 mg of bis-(triethylammonium) phosphate, prepared from dioxane diphosphate (7), in 20 ml of dry acetonitrile was added over a period of 2 hours. Stirring was continued overnight. The reaction was stopped by adding 40 ml of 0.1 N NH_4OH and 30 ml of ether. The ether phase was extracted twice with 40 ml portions of 0.1 N NH_4OH. The combined aqueous phases were concentrated in a rotatory evaporator at 40° under reduced pressure to a final volume of 122 ml containing 8×10^8 cpm (42% of the alcohol used).

The NH_4OH solution of the crude pyrophosphorylation product was applied to a 39 x 1.5 cm column of DEAE-Sephadex A-25, previously equilibrated with 10 mM Tris-HCl, pH 8.6. The same buffer (180 ml) was applied after the sample, followed by 250 ml of 0.1 M NH_4HCO_3 in 10 mM Tris-HCl, pH 8.6. The phosphorylated compounds were eluted by a linear gradient from 0.1 to 0.3 M NH_4HCO_3 in the same buffer (400 ml each) (9). Three radioactive peaks were collected. The fractions emerging between 0.20 and 0.23 M bicarbonate contained

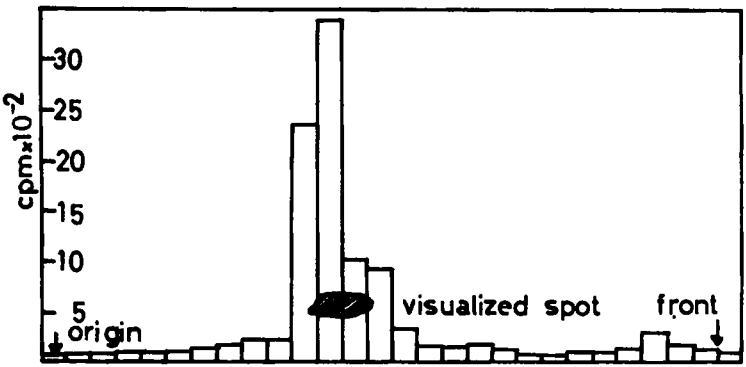


Fig. 1 PAPER CHROMATOGRAPHY OF 1-^{3}H-DIMETHYLALLYL PYROPHOSPHATE

A sample containing 0.0088 µmoles (22,800 cpm) of the pooled fractions eluted from a DEAE-Sephadex A-25 column between 0.20 and 0.23 M NH_4HCO_3 was applied with 0.05 to 0.1 µmoles of unlabeled DMAPP (prepared by the same procedure) on Whatman No 1 paper. The ascending chromatogram was developed (10) in n-propanol: NH_4OH:water (3:1:1). DMAPP was evidenced with molybdic acid (13) on one half of the paper band; the other half was cut into 1 cm wide strips and these were counted directly by conventional liquid scintillation spectrometry with an efficiency of 26%.

2.3×10^8 cpm (12.1% of the alcohol). Paper chromatography (10) of the pooled fractions showed a single phosphorus and radioactive spot with an R_f of 0.43 on paper chromatography (Fig. 1). The other radioactive peaks emerged at bicarbonate concentrations of 0.13 and and 0.27 M. Using the same solvent as in Fig. 1, each yielded on paper chromatography radioactive spots which may be tentatively identified as 1-^{3}H-3,3-dimethylallyl monophosphate and 1-^{3}H-3,3-dimethylallyl triphosphate by their R_f values (10) relative to DMAPP.

Characterization of 1-^{3}H-3,3-dimethylallyl pyrophosphate

Enzymic hydrolysis of the purified synthetic product with alkaline phosphomonoesterase from E. coli (Worthington Biochemical Corp., N.J.) plus potato apyrase (11) yielded a single radioactive peak on gas chromatography, coincident with carrier dimethylallyl alcohol (Fig. 2).

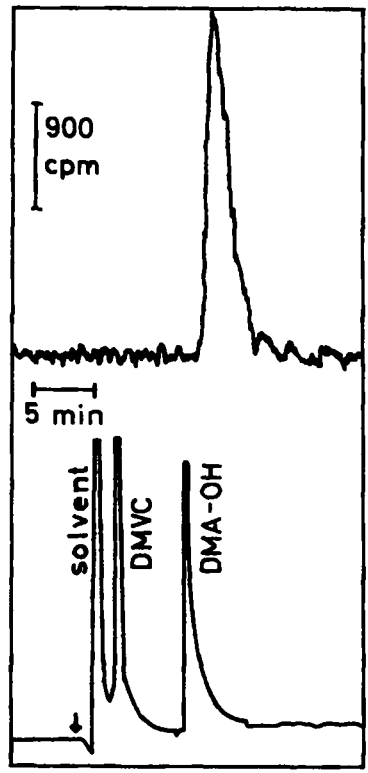


Fig. 2 GAS CHROMATOGRAPHY OF THE ALCOHOL MOIETY OF 1-^{3}H-DIMETHYLALLYL PYROPHOSPHATE

A sample containing 0.146 µmoles (380,000 cpm) of 1-^{3}H-DMAPP was treated with a mixture of potato apyrase and phosphomonoesterase from E. coli for 120 min at pH 8.6 (11). The prenols released were extracted with 1 ml of ether and an aliquot was injected into a gas chromatograph attached to a heated proportional counter (Biospan 4998, Nuclear Chicago). Gas chromatography was performed with the following program: 5 min isothermically at 70°, 5 min of a linear rise to 120° and then 20 min isothermically at 120°. For details of the column see text.
Upper tracing: Radioactivity.
Lower tracing: Carrier peak with added 3,3-dimethylallyl alcohol (DMA-OH) and dimethylvinylcarbinol (DMVC).

TABLE 1

PHOSPHORUS ANALYSIS OF 1-^{3}H-3,3-DIMETHYLALLYL PYROPHOSPHATE

Treatment prior to orthophosphate determination	H_3PO_4 found (μmoles)	Total phosphorus per sample corrected for inorganic orthophosphate (μmoles)
a) None	0.055	———
b) Incubation for 2 hours in N HCl at 37° and then with inorganic pyrophosphatase	0.355	0.300
c) Incubation for 30 min at 30° with potato apyrase	0.370	0.315

A sample of 0.160 μmoles (415,000 cpm) of DMAPP was used. Inorganic phosphate (a) was determined directly with the acid molybdate reagent (12). Another aliquot (b) was incubated for 2 hours at 37° in N HCl. The acid was neutralized with concentrated NaOH solution. Then 0.1 M Tris-HCl buffer at pH 8.6 plus 25 units of yeast inorganic pyrophosphatase (Sigma Chemical Co., St. Louis, Mo.) was added. Incubation was continued for 30 min at 37°, after which the sample was treated with the acid molybdate reagent. A third aliquot (c) was incubated with 50 units of potato apyrase in 0.1 M Tris-HCl buffer pH 8.6 containing 5 mM $CaCl_2$, and it was then treated with the acid molybdate reagent.

Table 1 shows a slight contamination of synthetic DMAPP with inorganic orthophosphate (experiment a). The acid reagent for phosphorus cleaves the DMAPP into rearranged alcohol plus inorganic pyrophosphate, which does not react with acid molybdate (12). Acid hydrolysis at 37° (experiment b) releases inorganic pyrophosphate (14) which is cleaved by inorganic pyrophosphatase and appears finally as orthophosphate. Potato apyrase releases orthophosphate from organic pyrophosphates (15) but does not attack the resulting phosphomonoesters, which are cleaved by the acid reagent (experiment c). Correction of the data in experiments b and c for orthophosphate shows a good agreement with the data calculated from specific radioactivity.

The synthetic product was thus concluded to be tritium labeled DMAPP. Its utilization in the biosynthesis of geraniol, nerol and farnesol by cell free enzyme systems from Citrus limonium and Citrus sinensis (1, 2) in the presence of unlabeled isopentenyl pyrophosphate confirmed this conclusion.

ACKNOWLEDGEMENTS:

This work was partially supported by grants from CONICYT (Chile), Comisión de Investigación Científica, Universidad de Chile, and the O.A.S. (Multinational Biochemistry Program). Consultation with Prof. C.A. Bunton, University of California, Santa Barbara, was possible through an Exchange Program funded by the Ford Foundation.

REFERENCES

1. Jacob, G., Cardemil, E., Chayet, L., Pont-Lezica, R. and Cori, O. - Phytochem. 11 : 1683 (1972).
2. George-Nascimento, C. and Cori, O. - Phytochem. 10 : 1803 (1971).
3. Colonna, A.O. and Gros, E.G. - J. Lab. Comp. 7 : 84 (1971).
4. Plieninger, H. and Immel, H. - Chem. Ber. 98 : 414 (1965).
5. Ogura, K., Nishino, T., Koyama, T. and Seto, S. - J. Am. Chem. Soc. 92 : 6036 (1970).
6. Holloway, P.W. and Popjak, G. - Biochem. J. 104 : 57 (1967).
7. Popjak, G., Cornforth, J.W., Cornforth, R.H., Ryhage, R. and Goodman, De W.S. - J. Biol. Chem. 237 : 56 (1962).
8. Dictionary of Organic Compounds. 2 : 1136 (1965). Eyre and Spottiswoode Publishers Ltd., London.
9. Cori, O. - Arch. Biochem. Biophys. 135 : 416 (1969).
10. Cramer, F. and Bohm, W. - Angew. Chem. 71 : 775 (1959).
11. Beytía, E., Valenzuela, P.,and Cori, O. - Arch. Biochem. Biophys. 129 : 346 (1969).
12. Fiske, C. and SubbaRow, Y.J. - J. Biol. Chem. 66 : 375 (1925).
13. Hanes, C.S. and Isherwood, F.A. - Nature. 164 : 1107 (1949).
14. Goodman, De W.S. and Popjak, G. - J. Lipid Res. 1 : 285 (1960).
15. Cori, O., Traverso-Cori, A., Tetas, M. and Chaimovich, H. - Biochem. Z. 342 : 345 (1965).