An Improved Synthesis of Isopentenyl Pyrophosphate

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1. Isopentenol was converted into isopentenyl phosphate with phosphoryl chloride in ether containing pyridine. 2. The isopentenyl phosphate reacted in 2-methylpropan-2-ol-water with morpholine and dicyclohexylcarbodi-imide to give isopentenyl phosphoromorpholidate. 3. The isopentenyl phosphoromorpholidate, with inorganic phosphate in pyridine containing tributylamine, gave isopentenyl pyrophosphate. The yield of pyrophosphate from monophosphate was 80–85% and the yield of pyrophosphate from isopentenol 40–60%. [^{14}C]-Isopentenyl pyrophosphate was prepared by this method. 4. The yield of isopentenyl pyrophosphate from isopentenyl phosphate was substantially improved, in comparison with the yields obtained by published methods via the phosphoramidate, by the use of the phosphoromorpholidate.

Isopentenyl pyrophosphate (3-methylbut-3-enyl pyrophosphate) is an important intermediate in the biosynthesis of polyisoprenoid compounds (for reviews see Clayton, 1965; Bloch, 1965). The ready synthesis of this compound in good yield, both with and without radioactive labelling, is thus of some interest. The successful use of phosphoromorpholidates in the synthesis of the pyrophosphate bond (Moffatt & Khorana, 1960, 1961; Roseman, Distler, Moffatt & Khorana, 1960) suggested the application of this method to the synthesis of isopentenyl pyrophosphate from isopentenyl phosphate. The expected improvement in yield of product has been realized.

Isopentenol (I) was converted into isopentenyl pyrophosphate (IV) by a three-stage synthesis depicted in Scheme 1. Isopentenyl phosphate (II) was prepared from isopentenol and excess of phosphoryl chloride in ether containing pyridine and was isolated as the dilithium salt (Foote & Wold, 1963) in 50–70% yield. The free acid was liberated from the dilithium salt by passage down an ion-exchange column, and was converted into isopentenyl phosphoromorpholidate (III) by refluxing in 2-methylpropan-2-ol-water (1:1, v/v) with a fourfold excess of morpholine and dicyclohexylcarbodi-imide. The isopentenyl phosphoromorpholidate reacted with excess of orthophosphoric acid in tributylamine-pyridine to give isopentenyl pyrophosphate (IV), which was isolated as the trilithium salt. The yield of pyrophosphate

from monophosphate was 80–85%. [^{14}C]Isopentenol was converted into [^{14}C]isopentenyl pyrophosphate by this method.

High-voltage paper electrophoresis on Whatman no. 1 paper with a pyridine-acetic acid buffer was found suitable for the separation of isopentenyl phosphate, isopentenyl phosphoromorpholidate, isopentenyl pyrophosphate and inorganic phosphate. The migration of these compounds to the cathode under the conditions described is shown in Table 1. The rate of migration of each compound is expressed as a fraction of that of inorganic phosphate (R_F). High-voltage paper electrophoresis was used to follow the course of the reactions and to determine the purity of the products.

DISCUSSION

Isopentenyl phosphoramidate has been used in the synthesis of isopentenyl pyrophosphate. In the first reported synthesis of isopentenyl pyrophosphate, isopentenyl phosphate was converted into the phosphoramidate with dicyclohexylcarbodi-imide and ammonia; the isopentenyl phosphoramidate formed was then treated with orthophosphoric acid to give isopentenyl pyrophosphate, which was isolated as its trisilver salt. The yield of pyrophosphate from monophosphate in this synthesis was 34% (Eggerer & Lynen, 1960; Lynen, Eggerer, Henning & Kessel, 1958).

In another synthesis of the pyrophosphate via the phosphoramidate, the latter compound was prepared from isopentenol in 23% yield by allowing the isopentenol to react with phenyl

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Scheme 1. Synthesis of isopentenyl pyrophosphate.

Table 1. *High-voltage paper electrophoresis of isopentenyl phosphate derivatives*

High-voltage paper electrophoresis was carried out on Whatman no. 1 paper in a pyridine-acetic acid buffer, pH 6.1, at 8 kv and 29–30 ma for 15–18 min. The detection reagent was an acid-ammonium molybdate spray, followed by heat and exposure to u.v. or spraying with ascorbic acid. The sample load was 10–20 μg .

Compound	R_{F1}
Isopentenyl phosphate (II)	0.75
Isopentenyl phosphoromorpholidate (III)	0.57
Isopentenyl pyrophosphate (IV)	1.06
Inorganic phosphate	1.00

phosphorodichloridate in dioxan containing quinine. Ammonia gas was passed through the cooled reaction mixture and the product was treated with base. The phosphoramidate was converted into the pyrophosphate by dioxan-phosphoric acid and the pyrophosphate was isolated as its cyclohexylammonium salt, in 27.5% yield (Yuan & Bloch, 1959). Foote & Wold (1963) described the synthesis of isopentenyl pyrophosphate from the monophosphate with diphenyl phosphorochloridate followed by anhydride-anion exchange effected with mono(tributylammonium) phosphate in pyridine. The yield of isopentenyl pyrophosphate, isolated as its trillithium salt, from the monophosphate, was 40%.

The advantage of phosphoromorpholidates over phosphoramidates in pyrophosphate bond synthesis has been well demonstrated by Moffatt & Khorana (1961). The present study further substantiates the improved yield of pyrophosphate obtained by the use of the phosphoromorpholidate.

EXPERIMENTAL

General. Pyridine (A.R.) and morpholine were refluxed over BaO and distilled with the exclusion of moist air. The

pyridine was stored over sticks of CaH_2 . Isopentenol was prepared from β -methylallyl chloride by published procedures (Yuan & Bloch, 1959). Dicyclohexylcarbodi-imide (British Drug Houses Ltd., Poole, Dorset) and LiOH (general purpose reagent; Hopkin and Williams Ltd., Chadwell Heath, Essex) were used without further purification.

Preparation of dilithium isopentenyl phosphate. Freshly distilled POCl_3 (4.1 ml.; 45 m-moles) and dry ether (6 ml.) were added to a 50 ml. round-bottomed flask equipped with a dropping funnel and cooled in an ice-salt bath at -10° to -15° . Isopentenol (2.58 g.; 30 m-moles), dry pyridine (3.63 ml.; 45 m-moles) and dry ether (6 ml.) were mixed in the funnel and added dropwise to the POCl_3 solution, with stirring. After the addition of the isopentenol the flask was tightly stoppered and left at -20° overnight.

The reaction mixture was passed through a sintered-glass disk into 10 g. of crushed ice, and the precipitate of pyridine hydrochloride on the sintered-glass disk was washed with the ether washings of the flask. LiOH soln. (4.32 g. of LiOH in 50 ml. of water) was added to the ether-ice mixture. The ether was removed by evaporation and the aqueous phase brought to pH 12. The precipitate of Li_2PO_4 was centrifuged down and washed with water. The combined supernatants were adjusted to pH 8 with dil. HCl and the preparation was freeze-dried.

The dry residue was washed with ethanol-ether (5:6, v/v) and was transferred with the solvent to a centrifuge tube. The precipitate was centrifuged down, washed once with ether and ethanol-ether, washed twice more with ether and dried *in vacuo* over P_2O_5 . The yield was 2.71 g. (51%) (Found: C, 33.6; H, 5.3; P, 17.2; $\text{C}_5\text{H}_9\text{Li}_2\text{O}_4\text{P}$ requires C, 33.7; H, 5.1; P, 17.4%).

Preparation of isopentenyl phosphoromorpholidate. Dilithium isopentenyl phosphate (890 mg.; 5 m-moles) was dissolved in water and passed through a Dowex 50 (H^+ form) column. Isopentenyl phosphate was washed off the column with water. 2-Methylpropan-2-ol (50 ml.), the column eluate (50 ml.) and purified morpholine (1.7 ml.; 20 m-moles) were brought into gentle reflux in a round-bottomed flask equipped with a dropping funnel and a reflux condenser. Dicyclohexylcarbodi-imide (4.12 g.; 20 m-moles) in 2-methylpropan-2-ol (20 ml.) was added dropwise from the funnel over 1 hr. The reaction mixture was refluxed for 4 hr. and left at room temperature overnight.

The dicyclohexylurea formed in the reaction was removed by filtration and the crystals were washed with 2-methylpropan-2-ol. The filtrate and washings were combined, the volume was reduced by evaporation *in vacuo* and the remaining water-alcohol mixture was extracted with ether. The ether extract was discarded and the aqueous phase evaporated to a gum. The gummy residue was washed three times with methanol and finally with ether. The product, a yellow gum, was stored over P_2O_5 in a vacuum desiccator. High-voltage paper electrophoresis of this product showed a single spot containing phosphate, with R_{F1} 0.57.

Preparation of trilithium isopentenyl pyrophosphate. The isopentenyl phosphoromorpholidate was dried by two evaporations of its solution in dry pyridine (20 ml.). Dry N_2 was led into the flask during the addition of fresh pyridine by using a three-way tap attached to the flask, an N_2 source and a high-vacuum line. Separately, aq. 88% H_3PO_4 (1.02 ml.; 15 m-moles) was dissolved in dry pyridine (10 ml.) containing tributylamine (3.6 ml.; 15 m-moles) and the mixture dried by removal of pyridine *in vacuo*. This step was repeated once, and then fresh pyridine was added and the solution transferred to the flask containing the dry phosphoromorpholidate. After the solution was mixed, the pyridine was removed, fresh dry pyridine was added and the flask securely stoppered.

After the mixture had stood for 50 hr. at room temperature (about 20°) the pyridine was removed *in vacuo*, the gummy residue was dissolved in water and LiOH soln. was added until the pH was 12. This solution was extracted with ether; the emulsion that formed was broken by centrifuging. Four layers separated, a precipitate of Li_3PO_4 , an aqueous layer, an interphase precipitate and an ether layer. The ether layer was discarded, the interphase precipitate and aqueous layer were decanted and the precipitate was washed with water. The combined water washings and aqueous layer were evaporated to dryness *in vacuo*, leaving a solid residue. This solid was extracted with ethanol-ether (5:6,

v/v), and the insoluble material collected by centrifuging. The pellet was washed twice with ether and dried *in vacuo* over P_2O_5 . The yield was 1.1 g. (83%) (Found: C, 22.9; H, 3.9; P, 21.8; $C_5H_9Li_3O_7P_2$ requires C, 22.8; H, 3.4; P, 23.4%).

High-voltage paper electrophoresis showed a spot with R_{F1} 1.06. The product contained a trace of isopentenyl monophosphate.

High-voltage paper electrophoresis. High-voltage paper electrophoresis was carried out on a Locarte apparatus. The buffer, pyridine-acetic acid, pH 6.1, was prepared by diluting pyridine (100 ml.) and acetic acid (8 ml.) to 251. with water. Whatman no. 1 paper was used throughout. Phosphates were detected on the chromatograms as blue spots by the acid-molybdate spray of Hanes & Isherwood (1949), followed by heating the paper at 90° until it was dry. The dry paper was exposed to u.v. light or sprayed with ascorbic acid.

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