Isopentenyl Pyrophosphate Isomerase from Liver

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Isopentenyl pyrophosphate isomerase (EC 5.3.3.2) was purified from extracts of pig liver by ammonium sulphate fractionation and by gel filtration. After about 20-fold purification the preparations were free of phosphatase and prenyltransferase (EC 2.5.1.1), the two enzymes that could have interfered with the assays. The isomerase has a distinct pH optimum at 6.0 and is activated by Mn^{2+} in preference to Mg^{2+} . The K_m value for isopentenyl pyrophosphate is $4 \times 10^{-6} M$. The equilibrium of the reaction favours the formation of dimethylallyl pyrophosphate. The reversibility of the isomerase reaction was demonstrated directly by the formation of isopentenyl pyrophosphate from dimethylallyl pyrophosphate. It is suggested that two prenyl isomerases might exist, one involved in the synthesis of *trans*- and another in the synthesis of *cis*-polyprenyl substances.

Isopentenyl pyrophosphate isomerase (prenyl isomerase; EC 5.3.3.2), which catalyses reaction (1), was first identified in yeast autolysates by Agranoff, Eggerer, Henning & Lynen (1960) and, more recently, in extracts of pig liver by Shah, Cleland & Porter (1965); both groups reported partial purification of the enzyme.

We confirmed the presence of prenyl isomerase in liver extracts during our work on the isolation of prenyltransferase (EC 2.5.1.1) and found that the two enzymes could be separated almost completely from one another by ammonium sulphate fractionation (Holloway & Popják, 1967). Several of our preparations of the isomerase, obtained by salt fractionation, were in fact free of the transferase and showed properties at variance with those described by Shah *et al.* (1965). Hence we decided to purify this enzyme further and to separate it from all enzymes that might interfere with its assays, enabling us to study its basic properties.

MATERIALS AND METHODS

Substrates. The preparations of [$1-^{14}C$]isopentenyl pyrophosphate and 3,3-dimethylallyl pyrophosphate were those described by Holloway & Popják (1967). The [$1-^{14}C$]isopentenyl pyrophosphate had a specific radioactivity of $0.069 \mu C/\mu mole$ and, according to analysis by high-voltage electrophoresis, contained 80% of the pyrophosphate and 20% of the monophosphate. An allowance was made in the calculations of substrate concentrations for the contamination of the substrate by the monophosphate, which is inactive with prenyl isomerase.

Reagents and procedures not mentioned specifically here were described by Holloway & Popják (1967).

Purification of prenyl isomerase. The starting material for the purification of the prenyl isomerase was the high-speed supernatant (S_{45}) of pig-liver extracts and the protein fraction (F_{70}) precipitated from this between 60 and 70% saturation with $(NH_4)_2SO_4$ at pH 7.5, as described by Holloway & Popják (1967). We have shown that the F_{70} fraction was almost free of prenyltransferase and that it contained nearly one-half of the total isomerase units found in the S_{45} fraction. The pellet of the precipitated F_{70} proteins, collected by centrifuging, was put directly into a dialysis sac and dialysed for 6 hr. against 10 mM-tris-HCl buffer, pH 7.7, containing 1 mM-2-mercaptoethanol; the dialysis fluid was changed twice. The dialysed solution was clarified by centrifuging at 40 000 *g* for 30 min.; the clear red supernatant (30–40 mg. of protein/ml.) retained its enzymic activity for several months when kept at -20° . Samples (10 ml. each) of the dialysed F_{70} solution were applied to a column (360 mm. $\times$ 25 mm.) of Sephadex G-200 (Pharmacia, Uppsala, Sweden) equilibrated with 10 mM-tris-HCl buffer, pH 7.7, containing 1 mM-2-mercaptoethanol. The column, which had a void volume of 67 ml., was eluted with the same buffer. The elution volume (V_e) for the isomerase was 170 ml. Haemoglobin was eluted just before the isomerase and served conveniently as a marker; the 20 ml. of effluent immediately after it contained the isomerase. The V_e for prenyltransferase from an identical column was 130 ml. (Holloway & Popják, 1967). The eluted solution of the isomerase, after addition of EDTA (final concn. 0.1 mM), was concentrated by 'dialysis' against solid sucrose overnight. The limp dialysis sac was then tightened and the sucrose was removed from the solution by dialysis against 10 mM-tris-HCl buffer, pH 7.7, containing 1 mM-2-mercaptoethanol but no EDTA. This concentrated solution of the isomerase (G-200-2 enzyme) was used in all studies described below, except for the determinations of the metal ion requirements of the enzyme. Such preparations retained their enzymic activity for several weeks when kept at -20° .

Removal of endogenous metal from the prenyl isomerase. A sample of the enzyme as eluted from the Sephadex column was dialysed for 2 hr. against 5 mM-tris-HCl buffer, pH 7.9, containing 1.0 mM-EDTA and 1 mM-2-mercaptoethanol, followed by dialysis for 2 hr. against the same medium but without EDTA. The metal ion requirement for this preparation of the isomerase was compared with that of the enzyme as eluted from the Sephadex column.

Assay of prenyl isomerase. The assay, coupled with prenyltransferase, described by Holloway & Popják (1967), was used only with crude preparations (S_{45}) of isomerase. In the present study we used a simple direct assay which was based, as is the assay of prenyltransferase, on the rapid hydrolysis of an allyl pyrophosphate by acid. The routine assays of isomerase contained in 1 ml. 100 μ moles of sodium maleate buffer, pH 6.0, 2 μ moles of $MnCl_2$, 0.5 μ mole of 2-mercaptoethanol, 40 m μ moles (6000 disintegrations/min.) of [^{14}C]isopentenyl pyrophosphate and 0.1 mg. of protein. This mixture was incubated for 5 min. at 37° and then acidified with 0.1 ml. of conc. HCl; 3,3-dimethylallyl alcohol and dimethylvinylcarbinol (1 mg. of each) were also added. The acidified mixture was kept at 37° for 30 min., after which it was saturated with NaCl and extracted four times with 1 ml. of light petroleum (b.p. 30–40°). The combined extracts were dried over anhydrous $MgSO_4$, diluted to 5 ml. with solvent and a sample was assayed for radioactivity. From the radioactivity of the extract and the known specific radioactivity of the isopentenyl pyrophosphate used as substrate, the amount of dimethylallyl pyrophosphate formed could be calculated.

The method of extracting the C_5 alcohols with light petroleum from the reaction mixtures saturated with NaCl was tested with [^{14}C]isopentenol: consistently only 65% of the alcohol added to a standard reaction mixture was recovered. When [^{14}C]isopentenyl pyrophosphate plus [^{14}C]dimethylallyl pyrophosphate were hydrolysed with intestinal alkaline phosphatase, again only 65–85% of the liberated alcohols was found in the petroleum extract (cf., e.g., Table 2). Although nearly quantitative extraction could be obtained with ethyl chloride at 0° (Cornforth, Cornforth, Popják & Yengoyan, 1966b) and particularly with toluene, the routine use of these solvents was cumbersome. We have not applied a correction factor in our assays for the inefficiency of extracting the C_5 alcohols with petroleum. For this reason, the activities of prenyl isomerase recorded are believed to be low by 15–35%.

Unit of enzyme. One unit of isomerase converts 1 m μ mole of isopentenyl pyrophosphate into 3,3-dimethylallyl pyrophosphate/min. under the conditions of the assay. Specific activities of the enzyme are given as units of enzyme/mg. of protein.

Assay of ^{14}C and gas-liquid radiochromatography. ^{14}C was measured in a Packard Tri-Carb scintillation spectrometer, as described by Holloway & Popják (1967). The column (9 ft. $\times$ 4 mm.) used in gas-liquid radiochromatography (Popják, Lowe & Moore, 1962) was packed with Celite (100–120 mesh) coated with 10% Carbowax 20M (Union Carbide Corp., New York, N.Y., U.S.A.). The column temperature was 85° for the analysis of isopentenol and dimethylallyl alcohol (cf. Figs. 4 and 5) and 185° for the analysis of nerolidol and farnesol.

Determination of the equilibrium of the prenyl isomerase reaction. A 5 ml. reaction mixture of the standard composition was set up with 22 units (1.4 mg. of protein) of

isomerase containing a total of 0.4 μ mole of [^{14}C]isopentenyl pyrophosphate (60 000 disintegrations/min.) plus isopentenyl monophosphate (15 000 disintegrations/min.). The mixture was incubated for 40 min. at 37°, 0.1 ml. samples being taken at 0, 5, 10, 20 and 40 min. intervals. Each sample was diluted with 0.9 ml. of water and acidified with 0.1 ml. of conc. HCl; 1 mg. of dimethylvinylcarbinol was also added to each sample. After 30 min. at 37° the samples (cf. Table 2; samples nos. 1–5) were extracted with light petroleum and the extracts analysed for radioactivity. The incubation mixture remaining after 40 min. (4.5 ml.) was heated to 90° for 30 sec.; it was then cooled and its pH adjusted to 8.5 with tris base, and 25 μ moles of $MgCl_2$ and 15 mg. of intestinal alkaline phosphatase (EC 3.1.3.1) were added. The phosphatase was allowed to act for 2 hr. at 37° before being inactivated by heat (90° for 30 sec.). A 0.1 ml. sample was added to 0.9 ml. of water, and the alcohols liberated by phosphatase were extracted in the usual way and their radioactivities determined (Table 2; sample no. 6). The rest of the heat-inactivated incubation mixture was transferred to a small distillation apparatus, 5 g. of solid $(NH_4)_2SO_4$ was added to it, and it was then distilled as described by Cornforth *et al.* (1966b). A sample (0.2 ml.) of the distillate (1.8 ml.) was assayed for radioactivity (Table 2, sample no. 7); the bulk of it was saturated with $(NH_4)_2SO_4$ and extracted at 0° three times with ethyl chloride (Table 2; sample no. 8). Octan-1-ol (7 μ l.), purified by preparative gas-liquid chromatography (Carbowax 20M column, 85°), was added to the ethyl chloride extract to prevent the loss of the microgram amounts of the C_5 alcohols during the subsequent evaporation of the ethyl chloride at room temperature. The residual octan-1-ol solution, after addition of unlabelled isopentenol and dimethylallyl alcohol (about 200 μ g. of each), was analysed by gas-liquid radiochromatography at 85° (Fig. 3). A control incubation, without enzyme, was treated in the same way.

Reversibility of prenyl isomerase reaction. A 5 ml. incubation sample of the standard composition was set up with 0.5 μ mole of 3,3-dimethylallyl pyrophosphate as substrate (instead of [^{14}C]isopentenyl pyrophosphate) with 24 units of enzyme. After 40 min. at 37° the mixture was treated with phosphatase, and the liberated alcohols were distilled, extracted with ethyl chloride and preserved by adding octan-1-ol, as described for the equilibrium experiment. The final octan-1-ol solution was analysed by gas-liquid chromatography at 85° on the Carbowax 20M column in a Pye Panchromatograph fitted with an argon-ionization mass-detector (W. G. Pye and Co., Cambridge).

RESULTS

Purification of prenyl isomerase. The purification of the prenyl isomerase is illustrated by the data in Table 1. The S_{45} preparations were, as shown before, grossly contaminated with prenyltransferase, which was still present in small amounts in the F_{70} fractions derived from some of the liver extracts (Holloway & Popják, 1967). The G-200-2 preparations, however, were free of the two enzymes that could have interfered with the assays of isomerase: (a) a phosphatase hydrolysing the [^{14}C]isopentenyl pyrophosphate to free isopentenol and inorganic phosphate, and (b) prenyltransferase.

Table 1. *Purification of prenyl isomerase from pig liver*

Details of purification procedures are described in the Materials and Methods section, where the symbols for the fractions are also defined.

Fraction	Total protein (mg.)	Specific activity	Total enzyme (units)
S ₄₅	22 000	1.5	33 000
F ₇₀	3 200	3.5	11 200
G-200-2	270	29.0	7 800

The presence of the phosphatase was excluded by the following experiment. The usual assay was set up with 4 units of isomerase and after a 5 min. incubation the enzyme was inactivated by heating the mixture at 90° for 30 sec. The mixture was cooled, saturated with sodium chloride, 1 mg. of unlabelled isopentenol was added to it and extracted with light petroleum. The extract contained no radioactivity. The aqueous phase was then acidified and after 30 min. at 37° was extracted again with light petroleum. This second extract was radioactive, 21.5 μ moles of acid-labile derivative having been formed from 40 μ moles of substrate. Hence the G-200-2 preparation was active with respect to isomerase, but contained no phosphatase.

The possible contamination of the G-200-2 preparations by prenyltransferase, which catalyses the synthesis of farnesyl pyrophosphate from dimethylallyl pyrophosphate and isopentenyl pyrophosphate (Holloway & Popják, 1967), was tested by an experiment in which high concentrations of enzyme and substrate were used followed by gas-liquid-radiochromatographic analysis of the products of the reaction. For this purpose a 2 ml. reaction mixture was set up containing 100 μ moles of tris-hydrochloric acid buffer, pH 7.9 (this is the optimum pH for liver prenyltransferase), 10 μ moles of magnesium chloride, 0.4 μ mole of [¹⁴C]isopentenyl pyrophosphate (60 000 disintegrations/min.), 0.5 μ mole of 2-mercaptoethanol and 20 units of isomerase. After 10 min. at 37° concentrated hydrochloric acid (0.2 ml.), carrier dimethylvinylcarbinol, linalool, geraniol, nerolidol and farnesol (1 mg. of each) were added and the mixture was incubated at 37° for a further 30 min. to complete the hydrolysis of allyl pyrophosphates. After saturation with sodium chloride, the alcohols were extracted with four 2 ml. portions of light petroleum. The extract, which contained 19 150 disintegrations/min., was concentrated to a small volume (about 50 μ l.). Samples of this concentrate were analysed by gas-liquid radiochromatography (cf. the Materials and Methods section) at a column temperature of 185°. Radioactivity appeared only in one fraction immediately at the end of the solvent

Fig. 1. Effect of pH on the activity of prenyl isomerase. For each determination standard 1 ml. incubation mixtures, containing 100 μ moles of buffer and 85 μ g. of protein (specific activity 22), were made. The buffers used were: ○, sodium succinate; □, sodium maleate; ▽, tris-maleate; Δ, tris-HCl.

Fig. 2. Activation of prenyl isomerase by Mn^{2+} (□) and Mg^{2+} (○). The determinations were made in 1 ml. incubation mixtures of the standard composition except for changes in the metal ions and their concentrations. The enzyme used was dialysed first against an EDTA-containing buffer, then against a buffer free of EDTA, as described in the Materials and Methods section. The specific activity of the enzyme as eluted from the Sephadex column and before the double dialysis was 22 with 5 mM- $MnCl_2$ and 16 with 5 mM- $MgCl_2$.

peak, i.e. in dimethylvinylcarbinol + dimethylallyl alcohol; the linalool, geraniol, nerolidol and farnesol peaks contained no trace of ¹⁴C. Another incubation mixture, identical with the one just described and to which 2.5 mg. (90 units) of purified prenyltransferase (cf. Holloway & Popják, 1967)

Table 2. *Equilibrium of prenyl isomerase*

Details of the experiment from which the data were obtained are described in the Materials and Methods section. The values recorded refer to the ^{14}C content of specimens of C_5 alcohols that would have been obtained by the particular method if the whole 5 ml. original incubation mixture had been processed. Experimental samples were incubated with isomerase, and control samples without isomerase.

Sample no.	Method of liberating and obtaining C_5 alcohol(s)	Incubation time (min.)	^{14}C content (disintegrations/min.)	
			Experimental samples	Control samples
1	H^+ ; extraction with petroleum	0	340	200
2	H^+ ; extraction with petroleum	5	22 300	—
3	H^+ ; extraction with petroleum	10	28 300	—
4	H^+ ; extraction with petroleum	20	34 600	—
5	H^+ ; extraction with petroleum	40	36 800	590
6	Phosphatase; extraction with petroleum	40	64 000	61 500
7	Phosphatase followed by distillation; determination of ^{14}C on aqueous distillate	40	56 500	62 000
8	Ethyl chloride extract of sample no. 7	40	47 700	48 600

was also added, was analysed in an identical way. The ^{14}C content of the petroleum extract (45 800 disintegrations/min.) indicated that 76% of the substrate was converted into acid-labile prenyl pyrophosphate. Gas-liquid radiochromatography of this extract showed ^{14}C only in nerolidol and in farnesol.

Properties of prenyl isomerase. The product of the reaction catalysed by prenyl isomerase from liver, established by Holloway & Popják (1967), was confirmed again (cf. Fig. 3).

We found, contrary to the report by Shah *et al.* (1965), that the isomerase from liver showed its maximum activity at pH 6.0 (Fig. 1) and that Mn^{2+} activated the enzyme more effectively than Mg^{2+} . With Mg^{2+} the activity of the enzyme, as eluted from the Sephadex column, was about 60% of that with Mn^{2+} . After dialysis against 1 mM-EDTA, followed by dialysis against EDTA-free medium (cf. the Materials and Methods section), the preferential activation of the isomerase by Mn^{2+} became particularly pronounced (Fig. 2).

The K_m value for isopentenyl pyrophosphate, as deduced from Lineweaver-Burk plots of the reciprocals of initial reaction velocities against the reciprocals of substrate concentrations, was $4.0 \times 10^{-6}\text{M}$.

We have tested only iodoacetamide among the possible inhibitors of the prenyl isomerase: pre-incubation of the enzyme with 1 mM-iodoacetamide for 5 min. at 37° caused 80% inhibition; complete inhibitions were observed at concentrations of 2–5 mM. In such experiments 2-mercaptoethanol was omitted from the reaction mixtures; its omission, however, caused no loss of activity in control incubations made in the absence of iodoacetamide at 37° for 5 min. Although 2-mercaptoethanol is needed to preserve the activity of the enzyme during storage, it is apparently not required

Fig. 3. Gas-liquid-radiochromatographic analysis of the product of the prenyl isomerase reaction for determination of its equilibrium. The details of the experiment, the results of which are given in Table 2, are described in the Materials and Methods section. The simultaneous recording of the output of the mass-detector and of the scintillation counter was taken with a multichannel recorder, the base lines of the two galvanometers being on opposite sides of the chart. (a) Analysis of sample no. 8 (Table 2) from control experiment without isomerase. Radioactivity coincided with the mass peak of isopentenol. (b) Analysis of sample no. 8 (Table 2) from experiment with isomerase. Radioactivity was associated with isopentenol (I-OH) as well as with dimethylallyl alcohol (DMA-OH).

for the stability of the enzyme during brief periods of incubation.

Equilibrium and reversibility of prenyl isomerase reaction. The results of the experiment, described in detail in the Materials and Methods section, for testing the equilibrium of the isomerase reaction are shown in Table 2. The results for the first five

samples suggest that probably no further amounts of dimethylallyl pyrophosphate would have been formed beyond 40 min. of incubation. Graphical examination of the results showed that the reaction had reached a plateau. That the reaction apparently stopped owing to an equilibrium state, and not because of inactivation of the enzyme, was shown by two other parallel experiments. In one of these fresh enzyme, and in the other a second measure of substrate, was added to the reaction mixture after 40 min. of incubation. In the latter the amount of dimethylallyl pyrophosphate rose within 5 min. as steeply as at the start of the experiment, whereas the addition of fresh enzyme was without effect.

Owing to the incompleteness and variability of the extraction of the C₅ alcohols from the reaction mixtures (cf. under 'Assay of prenyl isomerase' in the Materials and Methods section), it is not possible to calculate a precise value for the equilibrium constant of the reaction from the ¹⁴C content of sample no. 5 (Table 2). The gas-liquid-radio-chromatographic analysis of sample no. 8 should, however, give reliable information. The ratio of dimethylallyl alcohol to isopentenol in this sample was 1080:540 (Fig. 3). Since part of the isopentenol in this sample was derived from the isopentenyl monophosphate present in the substrate preparation, and which, though not a substrate for prenyl isomerase, is hydrolysed by phosphatase as efficiently as isopentenyl pyrophosphate and dimethylallyl pyrophosphate, it may be calculated that 324 counts/min. of the isopentenol fraction (i.e. 20% of the total radioactivity in dimethylallyl alcohol+isopentenol) in the specimen analysed must have come from the isopentenyl monophosphate. Thus the corrected ratio of dimethylallyl alcohol to isopentenol in the specimen should be 1080:216 = 5.

We have demonstrated the reversibility of the isomerase reaction, inferred previously from the incorporation of tritium into isopentenol when the reaction occurred in tritiated water (Agranoff *et al.* 1960; Shah *et al.* 1965), by the formation of isopentenol from dimethylallyl pyrophosphate. The gas-liquid-chromatographic analysis of the products of the experiment described in the Materials and Methods section is illustrated in Fig. 4. The three tracings shown are the analysis of: (a) the octan-1-ol used as final solvent for the C₅ alcohols; (b) the octan-1-ol solution of the extract of control experiment without isomerase; and (c) the octan-1-ol solution of the extract of incubation that contained the isomerase. On tracings (b) and (c) the peak marked DMA-OH is dimethylallyl alcohol; on tracing (c) the peak marked I-OH had the same retention volume as authentic isopentenol. The ratio of the areas of these two peaks in tracing (c) is 9:1, demonstrating again that dimethylallyl

Fig. 4. Gas-liquid-chromatographic analysis of the products of the action of prenyl isomerase on dimethylallyl pyrophosphate. The conditions of the experiment are described in the Materials and Methods section. Tracing (a) shows the analysis of the octan-1-ol used as final solvent for the C₅ alcohols. Tracing (b) is the record from the control experiment made without isomerase. Tracing (c) is the record from the experiment with isomerase. The retention volumes of peaks DMA-OH and I-OH correspond to those of authentic samples of dimethylallyl alcohol and isopentenol respectively. Only the rising limb of the octan-1-ol peak (O) is shown; peaks marked with arrows are due to impurities derived from the octan-1-ol.

pyrophosphate is the favoured product of the reaction at equilibrium, as found by Agranoff *et al.* (1960) and by Shah *et al.* (1965).

DISCUSSION

Although prenyl isomerase is a key enzyme in the synthetic sequence between mevalonate and farnesyl pyrophosphate, little has been learnt of its properties since its identification in yeast extracts by Agranoff *et al.* (1960). Indeed, the existence of this enzyme in liver has been inferred until recently only from the effect of iodoacetamide in a multi-enzyme system synthesising farnesyl pyrophosphate from mevalonate; in the presence of iodoacetamide the synthesis stopped at isopentenyl pyrophosphate (Cornforth *et al.* 1966b). This inference was based on the statement by Lynen, Agranoff, Eggerer, Henning & Möselein (1959), that prenyltransferase, which catalyses the synthesis of farnesyl

Scheme 1. Proposed reactions catalysed by a 'trans' (2) and 'cis' (3) prenyl isomerase. In the two reactions the hydrogen atoms eliminated from C-2 of isopentenyl pyrophosphate are epimeric; they are marked with a circle.

pyrophosphate from dimethylallyl pyrophosphate + isopentenyl pyrophosphate, or from geranyl pyrophosphate + isopentenyl pyrophosphate, was not inhibited by iodoacetamide. We have shown (Holloway & Popják, 1967) that this was not true for the prenyltransferase of liver; this enzyme is as severely inhibited by iodoacetamide as is prenyl isomerase. Hence the inhibition of the synthesis of farnesyl pyrophosphate from mevalonate, or from isopentenyl pyrophosphate, cannot be taken as conclusive proof for the existence of the isomerase in liver. Shah *et al.* (1965) have described the partial purification of prenyl isomerase from pig liver, but evidence as to the purity of their enzyme preparations has not been presented. The discrepancies in the properties of the liver isomerase as described by Shah *et al.* (1965) and by us here may be attributed to the impurity of the preparations of those workers. We found a distinct pH optimum for the isomerase at 6.0, and a preferential activation of the enzyme by Mn^{2+} . Shah *et al.* (1965) reported a broad pH optimum between 4.0 and 8.3, and an activation of the enzyme by Mg^{2+} rather than by Mn^{2+} . The differences could be explained if the preparations of Shah *et al.* (1965) had been contaminated by an acid phosphatase and by prenyltransferase.

The prenyl isomerase reaction is analogous to the prenyltransferase reaction, except that in the latter an allyl residue, not a proton, is being added to the terminal doubly bound methylene carbon atom of isopentenyl pyrophosphate (cf. reaction 1). It has been shown previously, with the aid of mevalonate labelled stereospecifically at C-4 with a hydrogen isotope, that the elimination of a proton from C-2 of isopentenyl pyrophosphate in the prenyltransferase reaction was stereospecific, according to the geometric configuration of the product formed. In an enzyme system that synthesises a *trans*-poly-prenyl substance the hydrogen eliminated from C-2

of isopentenyl pyrophosphate is the *R*-hydrogen, and when the product of the reaction has the *cis*-configuration the *S*-hydrogen is the one lost (Cornforth, Cornforth, Donninger & Popják, 1966a; Archer *et al.* 1966). It is not improbable that the same difference applies to the prenyl isomerase. Although *cis*- and *trans*-forms of dimethylallyl pyrophosphate can be distinguished only if its methyl groups are differentiated by isotopic labelling, it is nevertheless perfectly possible that two isomerases could exist, the as yet unknown one of the two converting isopentenyl pyrophosphate into dimethylallyl pyrophosphate by the elimination of the *S*-hydrogen of the precursor. Reactions (2) and (3) in Scheme 1 illustrate the difference between two dimethylallyl pyrophosphates formed from [4- ^{14}C]isopentenyl pyrophosphate by a 'trans' and a 'cis' isomerase. These two isomerases might exist in the latex of *Hevea brasiliensis*, which contains an enzyme system synthesising *trans*-polyprenyl substances (e.g. farnesyl pyrophosphate) and another synthesising *cis*-polyprenyls (rubber).

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