Tetrahedron Letters No.27, pp. 3133-3136, 1968. Pergamon Press. Printed in Great Britain.

SQUALENE BIOSYNTHESIS: ON THE MECHANISM OF THE REDUCTIVE DIMERIZATION OF FARNESYL PYROPHOSPHATE.

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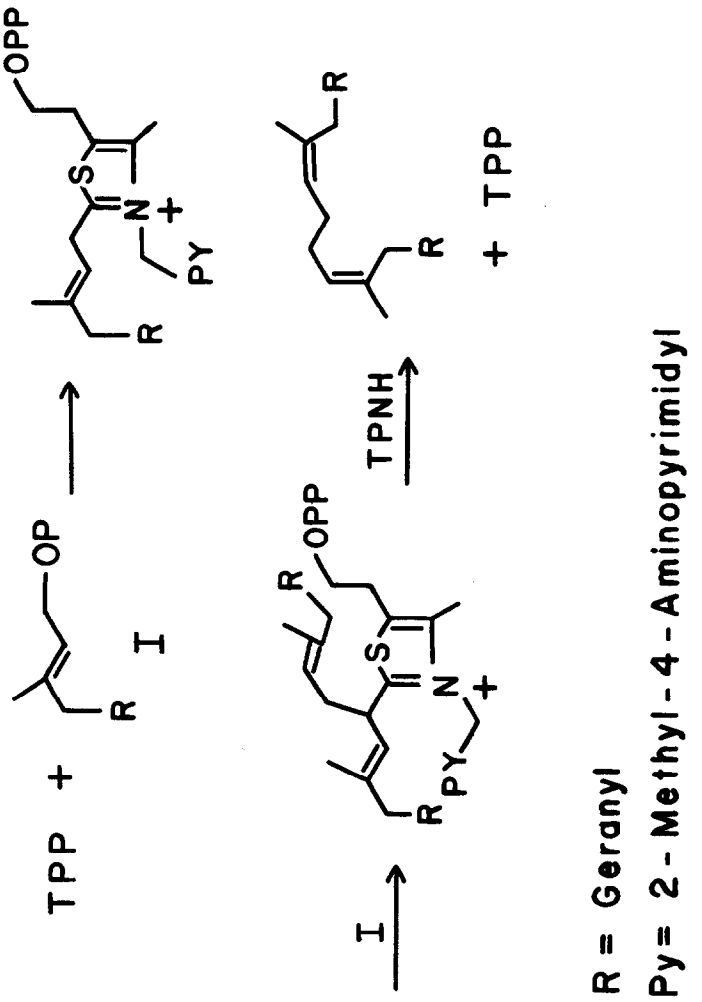
(Received in USA 29 February 1968; received in UK for publication 4 April 1968)

Although the mechanism of the head-to-tail polymerization of isoprenoid units has been thoroughly defined (1), the mechanism of the tail-to-tail and related "irregular" combinations of C-5 units still remains to be elucidated.

The tail-to-tail and "irregular" combinations, on theoretical grounds, would seem to require the services of a coenzyme, and recently we have obtained some information that indicates thiamine pyrophosphate (TTP) (3) may well be serving in this capacity. During the course of an investigation of thiamine chemistry, it came to our attention that the vitamin had been implicated in the biosynthesis of certain terpenoid compounds. In 1953, Starr and Saperstein (4) reported that Corynebacterium, in the absence of of thiamine, displayed colorless colonies; however, in the presence of the vitamin, the organism rapidly developed carotenoid pigments. This report has been recently reaffirmed by Larson and Pradip (5).

Based on these observations, we made a preliminary investigation into the effect of thiamine on the biosynthesis of squalene. The yeast enzyme preparation developed by Bloch, et al (6) was employed in these studies. Our initial approach to this problem was to remove coenzymes and cofactors from the preparation by dialyzing under alkaline conditions. After dialysis, the preparation was divided into two portions. To one portion, MVA, ATP, TPNH, Mg^{+2}, GSH, and TPP were added; to the second, all coenzymes and cofactors were added except TPP. The results of this experiment are described in the table.

Fig. 1



R = Geranyl

Py = 2-Methyl-4-Aminopyrimidyl

TABLE
EFFECT OF TPP ON THE CONVERSION
OF DL-2-C^{14}-MVA INTO SQUALENE

ADDITIONS (μm)	C. P. M. OF SQUALENE	
	A	B
TPP (0μm)	482	221
" (.5)	480	325

(A - dialyzed at pH of 7.1; B - dialyzed at pH of 8.2.)

Fleischmann's "Active Dry" Yeast was extracted with 0.066M $(NH_4)_2$ HPO_4 at 37° for 3 hours. The mixture was then centrifuged and the protein was precipitated with $(NH_4)_2$ SO_4 (70% saturation). The precipitated protein was centrifuged and resuspended in 0.02M phosphate buffer. This solution was then dialyzed for 8 hours at 4° against the same buffer at a pH of 7.1 and 8.2. Each experimental flask contained 1 ml of yeast extract, 2-C^{14}-MVA (2μm; 5 x 10^{-3} c/μm, DPNH (10μm), TPN (2μm), ATP (40μm), Mg SO_4 (40μm), and GSH (20μm). Each flask was incubated at 37° for 3 hours.

The dialyzed preparation was found to be reactivated only by the addition of either thiamine or TPP. We have concluded, therefore, that the tail-to-tail dimerization of farnesyl pyrophosphate is catalyzed by TPP[7], and have proposed the following reaction sequence (Fig. 1.);

ACKNOWLEDGEMENT

This investigation was supported in part by Public Health Service Research Grant AM-11488-01 from the National Institutes of Health.

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7. The biosynthesis of phytoene, in addition to squalene synthesis, can be described by the dimerization of geranylgeranyl pyrophosphate; this tail-to-tail dimerization, followed by an elimination reaction, would afford the C_{40} carotenoid precursor. The biosyntheses of the "irregular" terpenes, artemisia ketone and bakuchiol, are also readily explained on the basis of thiamine coenzymatic activity.