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MECHANISM OF BIOSYNTHESIS OF SQUALENE FROM SESQUITERPENOIDS

J. W. Cornforth

National Institute for Medical Research, Mill Hill, London

and G. Popják

Medical Research Council, Experimental Radiopathology Research Unit,

Hammersmith Hospital, London

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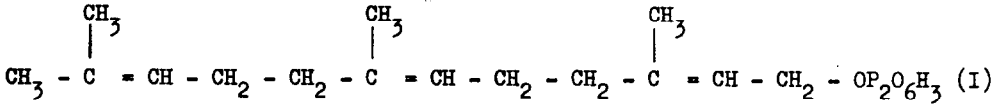
ALTHOUGH farnesyl pyrophosphate (I) has been shown to produce squalene when incubated with yeast cell particles and reduced triphosphopyridine nucleotide,[1] the detailed mechanism of this change is still unknown. The two hypotheses previously advanced[1,2] are incompatible with the new data[2] obtained by using hydrogen isotopes to study the biosynthesis of squalene from mevalonic acid.



(I)

We now propose an alternative scheme based on the discovery of acid-labile derivatives, presumably pyrophosphates, of both nerolidol and farnesol in a liver enzyme system after incubation with mevalonate.[3]

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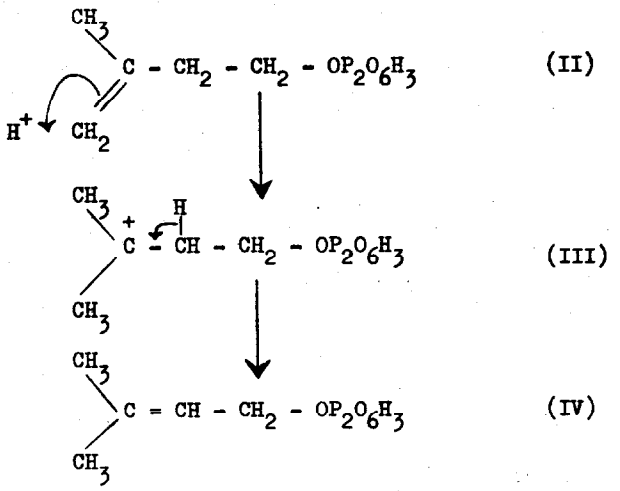
[1] F. Lynen, H. Eggerer, U. Henning and I. Kessel, Angew. Chem. 70, 739 (1958).

[2] H. C. Rilling and K. Bloch, J. Biol. Chem. 234, 1424 (1959).

The association of C_5 units, derived from mevalonate, to polyisoprenoid structures is thought to begin with the enzymic isomerixation[4] of isopentenyl pyrophosphate (II) to 3:3-dimethylallyl pyrophosphate (IV).



This process can be formulated chemically as the electrophilic addition of a proton to the reactive terminal double bond in (II). The resulting cation (III) then stabilizes itself by expulsion of a different proton to give (IV). Variants of this basic mechanism can be written: for example, fusion of the two steps into a concerted electron-shift, or (as Lynen suggested[4]) an intermediate neutralization of the cation (III) by attachment of a sulphydryl anion.

3:3-Dimethylallyl pyrophosphate, in common with other substances in which a potentially anionic group is in the α-position to a double bond, can

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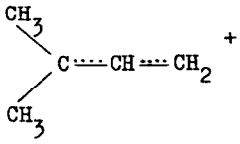
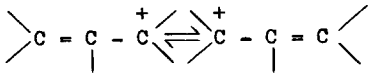
[3] G. Popjak, Tetrahedron Letters No. 19, 19 (1959).

[4] B. W. Agranoff, H. Eggerer, U. Henning and F. Lynen, J. Amer. Chem. Soc. 81, 1254 (1959).

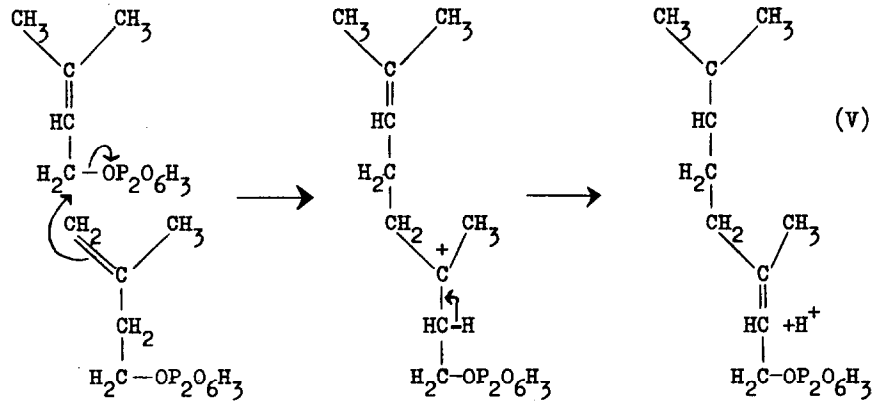
be expected to lose the pyrophosphate ion rather readily. The resulting electron deficient species,



, is partially stabilized by mesomerism between two equivalent forms (

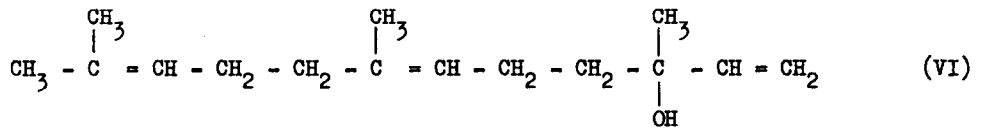


). A molecule of isopentenyl pyrophosphate could be attacked by this cation as already described for attack by a proton. The product is geranyl pyrophosphate (V), itself an allylic derivative which by loss of pyrophosphate and attack on a third molecule of isopentenyl pyrophosphate can produce farnesyl pyrophosphate (I). In these condensations variants in the timing of the electron-shifts can again be formulated; the mechanism as set forth is, however, essentially similar to that postulated by Lynen[1] and by Bloch.[2]



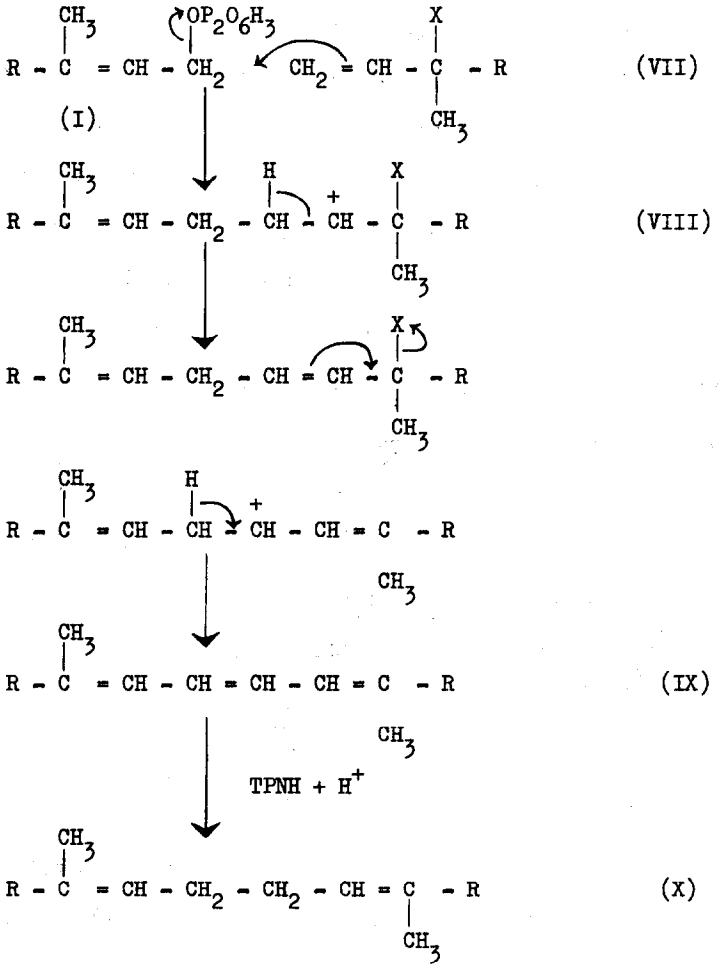
(V)

The presence in liver-enzyme system, after incubation with mevalonate, of a hydrophilic acid-labile derivative of nerolidol (VI), can readily be



(VI)

explained as arising from farnesyl pyrophosphate by anionotropy to give the derivative $R-C(CH_3)(X)-CH=CH_2$ (cf. also ref.[3]) The group X is presumably, but for the present argument not necessarily, pyrophosphate.



In the nerolidyl derivative (VII) and farnesyl pyrophosphate (I) we have two reactants which could condense with each other as isopentenyl

pyrophosphate is presumed to do with 3:3-dimethylallyl pyrophosphate or with geranyl pyrophosphate. The intermediate cation (VIII) could then become stabilized by the loss, in successive stages, of a proton, the anion X^-, and another proton, to yield dehydrosqualene (IX), reduction of which to squalene (X) is presumed.

The significant points of the chemical mechanism of squalene biosynthesis as outlined are: (a) that the process is not a concerted reaction, but proceeds in steps with well defined stable intermediates; (b) that during isomerization of isopentenyl pyrophosphate there is an uptake of one proton in the terminal methylene group and that this proton appears finally in one of the terminal methyl groups at each end of squalene which means the entry into each molecule of squalene of two protons not contained originally in mevalonic acid; (c) that there are no reductive steps involved in the synthesis of geranyl or farnesyl pyrophosphate; (d) that farnesyl pyrophosphate and nerolidyl derivative are the two sesquiterpenoids condensing to the symmetrical dihydrotriterpene squalene, a stable intermediate being dehydrosqualene; (e) that during stabilization of the condensation product of the farnesyl and nerolidyl derivatives elimination of two protons, originally attached to C-5 of mevalonate, occurs and (f) that the final step is a reduction, introducing into squalene two further hydrogen atoms not contained originally in mevalonic acid.

Although further experimental work is needed to establish each of these points unequivocally, supporting evidence for this scheme is already available. The finding in a single liver enzyme preparation of the formation from mevalonate of derivatives of dimethylallyl alcohol, of geraniol, of farnesol and of nerolidol[3] shows that the synthesis of the C_{15} derivatives

is not a concerted reaction, but occurs in steps; for the utilization of the C_{15} compounds for squalene synthesis enzymes attached to microsomes are needed,[1,3] all of which is in accord with postulate (a). The most relevant information concerning postulates (b), (e) and (f) comes from the recent work of Rilling and Bloch[2] who (i) analysed the distribution of deuterium in squalene biosynthesized from [5-$^{2}H_{2}$-2-^{14}C]mevalonate by a yeast autolysate, and (ii) determined the extent and position of deuterium and tritium labelling in squalene biosynthesized from [2-^{14}C]mevalonate in the presence of $^{2}H_{2}O$ and $^{3}H_{2}O$ respectively. They found that out of the 12 possible deuterium atoms which might have been incorporated into squalene from [5-$^{2}H_{2}$] mevalonate only 10 were contained in the molecule and that the two which disappeared were lost from the two central carbon atoms of squalene. Thus postulate (e) is satisfied. The experiments with heavy water showed on the other hand that during squalene synthesis from mevalonic acid 4 atoms of hydrogen were taken up from the reaction medium, 2 of these were located at the two central carbon atoms and the remainder (which suffered some loss during chemical degradation) in the two terminal isopropyl-groups of squalene. These results support the requirements of points (b) and (f). It should be pointed out, however, that the uptake of hydrogen from the water of the reaction medium during a reductive process depends on the nature of the reducer. Since both in the yeast[1] and in the liver enzyme system[3] reduced pyridine nucleotides are needed for the utilization of farnesyl-pyrophosphate (or of farnesyl- and nerolidyl-pyrophosphate) for squalene synthesis, one might have expected in the experiments of Rilling and Bloch the uptake of only one atom of hydrogen (proton) from the water of the incubation medium instead of the observed two into the centre of

squalene if the reducer was TPNH + H^+, or DPNH + H^+. On the other hand in the experiment quoted a crude enzyme system, fortified with DPN (not DPNH) was used and thus the possibility exists that DPN^{2}H was formed by some indirect mechanism. Some incorporation of deuterium from DPN^{2}H, generated with [1-$^2H_2$] ethanol and alcohol dehydrogenase, into squalene during its biosynthesis from mevalonate was noted in preliminary experiments by Rilling and Bloch,[2] but the values obtained were too low and variable to decide whether DPNH was the immediate reducing agent, or whether it functioned in their system by reducing another coenzyme. In support of the possible existence of dehydrosqualene as an intermediate it might be relevant to mention that in the carotenoid series, in phytoene, the conjugated triene structure is present in the six central carbon atoms.