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ENZYMATIC SYNTHESIS OF SQUALENE ANALOGS FROM GERANYL PYROPHOSPHATE ANALOGS

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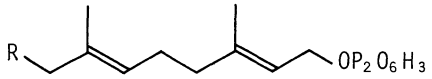
8-*n*-Butylgeranyl pyrophosphate and 10,11-dihydrofarnesyl pyrophosphate act as substrates for the "tail-to-tail" condensation catalyzed by pig liver microsomal enzyme, yielding the corresponding squalene analogs.

It has been shown that two homofarnesyl pyrophosphates, 12-methyl- and 12'-methylfarnesyl pyrophosphate act as substrates in the "tail-to-tail" condensation catalyzed by liver microsomal enzyme to give the corresponding squalene homologs whereas neither 12-ethyl- nor 12'-ethylfarnesyl pyrophosphate is accepted.[1,2]

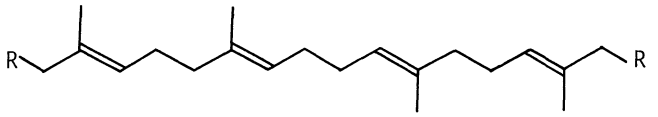
We now report the finding that the terminal double bond of the farnesyl moiety is not essential for the enzymatic condensation so that certain homologs of the geranyl type can also be substrates.

Radio-labeled homologs of geranyl pyrophosphate (1 ∿ 4) were synthesized from [^{14}C]-isopentenyl pyrophosphate and the appropriate allylic pyrophosphate by the action of farnesyl pyrophosphate synthetase according to a method similar to that reported previously,[1,3] and their reactivities were examined for the "tail-to-tail" condensation. The incubation mixture contained, in a final volume of 1 ml, 5 μmol of $MgCl_2$, 100 μmol of phosphate buffer, pH 7.4, 30 μmol of nicotinamide, 1 mg of NADPH, 10 μmol of KF 2 x 10^4 dpm of [^{14}C]-labeled homolog (specific activity, 1.2 Ci/mol) of geranyl pyrophosphate to be examined, and 0.2 ml of doubly washed microsomes prepared from pig liver homogenate.[4] The incubation was carried out anaerobically at 37° for 2 hr, and the non-saponifiable fraction was extracted with light petroleum, and was subjected to TLC.

Two out of the four compounds tested were found to be enzymatically active to give radioactive materials which behaved similarly to squalene. The materials obtained from 8-*n*-butylgeranyl pyrophosphate (2) and 10,11-dihydrofarnesyl pyrophosphate (4) showed R_f values of 0.61 and 0.63, respectively, on silica gel TLC with *n*-hexane in which squalene had an R_f of 3.2. In order to confirm the structure, the incubation was carried out in a 20-times scale using non-radioactive substrates, and the hydrocarbon fraction was purified by TLC, and was analyzed by gaschromatograph-mass spectrometry (GC-MS). The GC-MS with a column of OV-1 showed that the product derived from 2 emerged at a retention volume of 0.84 relative to that of squalene, exhibiting a parent ion at m/e 386 corresponding to $C_{28}H_{50}$. The material from 4 also showed a slightly shorter retention volume and gave a parent ion at m/e 414 corresponding to $C_{30}H_{54}$. As shown in Table 1, the fragmentation patterns of these two materials which are closely analogous to each other support the expected structures, 5 and 6.



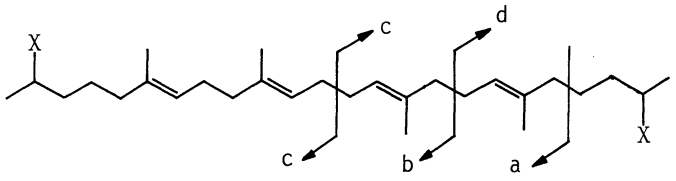
1 : R = n-C_3H_7
2 : R = n-C_4H_9
3 : R = n-C_5H_{11}
4 : R = iso-C_5H_{11}



5 : R = n-C_4H_9
6 : R = iso-C_5H_{11}

Table 1. Partial Mass Spectra of 5 and 6

Species	5 (X = H) m/e	5 (X = H) Rel. abundance	6 (X = CH_3) m/e	6 (X = CH_3) Rel. abundance
M	386	22.4	414	5.6
a	329	5.6	343	3.4
[M − $C_7H_{14}R$]	287	28.2	301	8.3
b	261	16.4	275	4.1
c	193	37.6	207	4.1
d	125	79.0	139	16.2
C_6H_{11}	83	84.5	83	100
C_5H_9	69	100	69	76.0



The rate of conversion of 2 and 4 to the corresponding squalene analogs were 28 and 22% of that of the natural substrate, farnesyl pyrophosphate to squalene. On the other hand, neither 1 nor 3 was enzymatically active, suggesting that the requirement of the enzyme is stringent with respect to the chain-length rather than the terminal double bond.

References

1) K. Ogura, T. Koyama and S. Seto, *J. Amer. Chem. Soc.*, 94, 307 (1972).
2) A. Polto, G. Popják and T. Parker, *J. Biol. Chem.*, 247, 3464 (1972).
3) K. Ogura, T. Nishino, T. Koyama and S Seto, *J. Amer. Chem. Soc.*, 92,6036 (1970).
4) D.S. Goodman and G. Popják, *J. Lipid Res.*, 1, 286 (1960).

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