SYNTHESIS OF 1-T_2-2-C^{14}- AND OF 1-D_2-2-C^{14}- TRANS-TRANS-FARNESYL PYROPHOSPHATE AND THEIR UTILIZATION IN SQUALENE SYNTHESIS

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We have presented evidence recently indicating that during biosynthesis of squalene from farnesyl pyrophosphate (FPP) by liver microsomes one hydrogen atom attached to C-1 of one of the two FPP molecules condensing to squalene is exchanged for a hydride ion derived from TPNH (Popják et al. 1961). This conclusion was drawn from the examination of the labeling of squalene biosynthesized from mevalonate-2-C^{14}-5-D_2 and from the observation that during synthesis of squalene from biosynthetically prepared C^{14}-FPP by liver microsomes in the presence of THO or T-labeled TPNH nearly one atom of labeled H was transferred to squalene only from the labeled TPNH and not from THO. We felt that further formal proof for the one exchanged H-atom referred to was required. We have therefore synthesized 1-T_2-2-C^{14}- and 1-D_2-2-C^{14}-trans-trans-farnesyl pyrophosphate from labeled trans-trans-farnesols and tested the preparations in biosynthetic experiments.

First, methyl farnesoate-2-C^{14} (3,7,11-trimethyldodeca-2,6,10-trienoate) was synthesized from trans-geranylacetone (1.94 g.) and methyl bromoacetate-2-C^{14} (1.53 g., sp. a. about 0.15 μc./μmole) by the Reformatsky reaction, which gave methyl 3-hydroxy-3,7,11-trimethyldodeca-6,10-dienoate (1.86 g.). The ester of this hydroxy acid was dehydrated with $POCl_3$ (1.6 ml.) in pyridine (16 ml.) during 3 days at room temperature. After the usual extraction procedures 1.68 g. of methyl farnesoate-2-C^{14} was obtained which, according to gas-liquid radiochromatographic analysis (Popják et al. 1959), consisted of methyl cis-

trans-farnesoate (45%) and trans-trans-farnesoate (43%) and of some other unidentified radioactive impurities (12%). The cis-trans and trans-trans components were separated by preparative gas-liquid chromatography at 197° using a 6 foot x 1 inch column packed with Celite 545 coated with 10% "Apiezon-L" vacuum grease (cf. Popják and Cornforth, 1960). The purity of the separated methyl trans-trans-farnesoate-2-C^{14} was 95% by gas-liquid chromatography and was considered sufficient for further work.

Trans-trans-farnesol-1-T_2-2-C^{14}, and 1-D_2-2-C^{14} were prepared by reduction of methyl trans-trans-farnesoate-2-C^{14} with T-labeled $LiAlH_4$ and $LiAlD_4$ respectively. Experimental details are given for the T-labeled compound.

Methyl trans-trans-farnesoate-2-C^{14} (40.8 mg.) was diluted with unlabeled ester (367.6 mg.) (synthesized and purified as the C^{14}-compound) and added in ether (5 ml.) to a solution of tritio-$LiAlH_4$* (10 mg. $LiAlT_4$ + 85 mg. $LiAlH_4$) in ether (10 ml.) at -30° during 30 minutes, the reaction mixture being stirred at -30° for a further 90 minutes. After the usual working up and exchange of labile H with ethanol and H_2O, 369 mg. of farnesol-1-T_2-2-C^{14} were obtained, 95% of which consisted of the trans-trans-isomer (gas-liquid chromatography).

Trans-trans-farnesol-1-D_2-2-C^{14} was made similarly from 506 mg. of methyl trans-trans-farnesoate-2-C^{14} and 422 mg. of $LiAlD_4$ (99.2% D); yield 425 mg. of trans-trans-farnesol-1-D_2-2-C^{14} (93% pure). The labeled farnesols were phosphorylated according to the procedure of Cramer and Böhm (1959); 333 mg. of the T-farnesol yielded 100 mg. of the monophosphate (cyclohexyl-ammonium salt) and 301 mg. of the pyrophosphate (Li-salt); 403 mg. of the D-farnesol gave 87 mg. of the monophosphate and 127 mg. of the pyrophosphate together with some further unidentified products. The synthetic FPP preparations (pyrophosphate-P : 14.0%, theoretical for Li_3-salt 15.5%; total-P 14.9%) behaved as the biosynthetic specimens in that they cleaved rapidly

* Specific activity unknown as the suppliers informed us after the experiment was completed that they had reasons to believe that the preparation was unsatisfactory. The radioactive yield of the T-labeled farnesol was approximately 1/100th of that anticipated.

at room temperature in acid solution (pH < 2) releasing inorganic pyrophosphate with concommittant allylic rearrangement of most of the farnesol to nerolidol (Lynen et al. 1958; Goodman and Popják 1960). Hydrolysis with intestinal alkaline phosphatase at pH 8.7, on the other hand, gave trans-trans-farnesol (85%), cis-trans-farnesol (5.3%), nerolidol (0.55%) and four further small components not identified.

Both the 1-D_2-2-C^{14} and the 1-T_2-2-C^{14}-FPP preparations were tested as substrates in the anaerobic squalene synthetase system of rat liver microsomes in standard 1 ml. incubations described in detail previously (Goodman and Popják, 1960, Popják et al. 1961) and were converted to squalene. The 1-T_2-2-C^{14}-farnesol contained 89,100 d.p.m. of T and 30,050 d.p.m. of C^{14} per μmole, and the squalene biosynthesized from the 1-T_2-2-C^{14}-FPP 2330 d.p.m. of T and 1018 d.p.m. of C^{14}. Calculating the yield of squalene from the C^{14} counts, there were synthesized 1018/(30,050 x 2) = 0.0169 μmoles of squalene, within the range of the biosynthetic activity usually observed in 1 ml. incubations (cf. Popják et al. 1961). It is obvious that the T/C^{14} ratio in the FPP and squalene biosynthesized from it were different. Taking this ratio in the FPP to have been 1.00, the ratio in the squalene was < 0.77 indicating the loss of about one labeled H-atom from one of the two FPP molecules (the theoretical ratio for the loss of one labeled H-atom out of four is 0.75) in complete agreement with our previous results (Popják et al. 1961). It is highly probable that the loss of the one hydrogen atom occurs from an intermediate formed after the condensation of two sesquiterpenoids (cf. Popják et al. 1961) and that the removal of the H-atom is stereo-specifically determined and hence no "isotope effect" should be discernible as the two H-atoms of C-1 of FPP must have been labeled to the same extent.

The data from the experiment with 1-D_2-2-C^{14}-FPP are relevant at present only to the extent that the chemically synthesized specimen (like the 1-T_2-2-C^{14}-FPP) acted as substrate for the biosynthesis of squalene (in a 1 ml. incubation 0.04 μmole squalene being synthesized) providing a formal proof for

the identity of the immediate precursor of squalene deduced previously from the study of trace (or μmole) amounts of biosynthetic material (Lynen et al., 1958; Goodman and Popják, 1960).

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