Artificial Substrates in Squalene and Sterol Biosynthesis*

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SUMMARY

Trans-[1-\(^{3}H\)]3-methylpent-2-enyl pyrophosphate and [1-\(^{14}C\)]isopentenyl pyrophosphate were incubated with 10,000 X g supernatants of rat liver homogenates. Analyses of the unsaponifiable material extracted from the incubations by gas-liquid radiochromatography and mass spectrometry revealed the synthesis not only of [\(^{14}C\)]squalene but also of [\(^{3}H\), \(^{14}C\)]1-methylsqualene and of [\(^{3}H\), \(^{14}C\)],24-dimethylsqualene. The sterol fraction of the unsaponifiable extracts contained two components labelled with \(^{3}H\) and \(^{14}C\), the \(^{3}H\):\(^{14}C\) ratios of which were identical with the isotope ratios of the 1-methylsqualenes and which had retention volumes in gas-liquid chromatography expected of 27-methylcholesterol and of 27-methylnanosterol. The sterol fractions did not contain components that might have been derived from the cyclization of 1,24-dimethylsqualene.

It is suggested that 1-methylsqualene is epoxidized enzymically only at its isopropylidene end, but not at its isobutylidene end.

It has been shown that an analog of geranyl pyrophosphate (6,7-dihydrogeranyl pyrophosphate) and several homologs of 3,3-dimethylallyl (3-methylbut-2-enyl) pyrophosphate (cis- and trans-3-methylpent-2-enyl, 3-ethylpent-2-enyl, and cis- and trans-3-methylhex-2-enyl pyrophosphate) acted as condensing partners with isopentenyl (3-methylbut-3-enyl) pyrophosphate in the reaction catalyzed by prenyltransferase of liver (EC 2.5.1.1), the products of synthesis being 10,11-dihydrofarnesyl pyrophosphate (1) and the appropriate homo- and bishomofarnesyl pyrophosphates (2). Ogura et al. (3), using the prenyltransferase of pumpkin, confirmed these observations and showed that even trans-3-methyldec-2-enyl pyrophosphate reacted with isopentenyl pyrophosphate to produce a homolog of geranyl pyrophosphate.

It became of interest to examine whether squalene synthetase could accept as substrate a homofarnesyl pyrophosphate and, if so, whether the resulting homo- and bishomosqualenes might be cyclized to sterol.

In order to explore these possibilities we have incubated trans-

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pared as described previously (2) except that the intermediary product of synthesis, methyl trans-3-methylpent-2-enoate, separated from the cis isomer by preparative gas-liquid chromatography, was reduced to the alcohol with LiAlH₄. The specific activity of the pyrophosphate ester was 6 Ci per mole.

**Enzyme Preparations**—The 10,000 × g supernatants (S₁₀) of rat liver homogenates were made in 0.1 M Tris-HCl buffer containing 5 mM MgCl₂, 30 mM nicotinamide, and 10 mM KF as described (4) for the study of total sterol biosynthesis from mevalonate. The S₁₀ preparations were kept frozen at -20° for 4 to 5 days before use. The conditions of incubation are described in the footnote to Table I.

**Extraction, Fractionation, and Analysis of Products Synthesized**—The incubation mixture was hydrolyzed with 2 N KOH in the presence of 50% ethanol at 70° for 1 hour. The neutral material was extracted with light petroleum (b.p. 40-60°) and divided into a hydrocarbon and crude sterol fraction by chromatography on neutral alumina (4) with light petroleum followed by acetone-diethyl ether (1:1, v/v). The acetone-diethyl ether eluate was reduced to the alcohol with LiAlH₄. The specific activity of the pyrophosphate ester was reduced to the alcohol with LiAlH₄. The specific activity of the pyrophosphate ester was 6 Ci per mole. The neutral material was extracted with light petroleum (b.p. 40-60°) and divided into a hydrocarbon and crude sterol fraction by chromatography on neutral alumina (4) with light petroleum followed by acetone-diethyl ether (1:1, v/v). The acetone-diethyl ether eluate could contain not only sterols, but also prenols.

The hydrocarbon fraction was first analyzed by the gas-liquid radiochromatographic technique of Popják et al. (7) devised for the simultaneous assay of ³H and ¹⁴C. The scintillation counter (7) was connected to a Packard two-channel rate meter (model 3022, Packard Instrument Co., Ambar Industries Inc., LaGrange, Ill.), the output of the rate meter and of the gas chromatograph mass detector (thermal conductivity cell) being recorded on a three-channel recorder. The efficiencies of ¹⁴C and ³H counting in this gas-liquid radiochromatography system were 38 to 40% and 14 to 16% during the simultaneous assay of the two isotopes, respectively. The main feature of this system is that the ¹⁴C and ³H counts are displayed on different channels, neither of which is "contaminated" by contribution from the other isotope (see Fig. 1).

The same fraction was analyzed also in a gas-liquid chromatograph combined with a mass spectrometer as described (8). The same gas-liquid chromatographic column was used in both analyses: a coiled stainless steel tube (180 × 0.3 cm) filled with 10% Dexsil-300 (Olin Corporation, New Haven, Conn.) on Chromosorb W. The gas-liquid chromatographic analyses were made at 350-290°. Background mass spectra were taken both before and after the completion of the gas-liquid chromatography-mass spectrometry analyses. The background spectra, of remarkably low intensity for a "bleed" from a column with a 10% stationary phase, were subtracted from those of the specimens.

The crude sterol fraction from Experiment 1 was analyzed by the gas-liquid radiochromatography method after addition of unlabelled desmosterol, lanosterol, and dihydrodesmosterol to the specimen and conversion of the alcohols to trimethylsilyl ethers (9). In Experiment 2 the 3β-hydroxysterols were first precipitated as the digitonides which were then decomposed with pyridine. The sterols were extracted by partitioning between diethyl ether and dilute HCl and were examined as in Experiment 1 by the gas-liquid radiochromatography method.

**Radioactivity Measurements**—The ³H and ¹⁴C content of every specimen at each step of fractionation was determined in a Packard Tri-Carb scintillation spectrometer.

## Results

Both the [1-³H₂]3-methylpent-2-yl and the [1-¹⁴C]isopentenyl pyrophosphates were used for the synthesis of unsaponifiable products. In the two experiments recorded in Table I, 24.3% and 14.7% of the ³H and 75.8% and 72.2% of the ¹⁴C of the two substrates, respectively, were found in the unsaponifiable extract, most of the ³H appearing in the hydrocarbon fraction and about one-half of the ¹⁴C in the crude sterol fraction. In Experiment 2 the sterol digitonides accounted for 40,172 dpm of ³H and 47,874 dpm of ¹⁴C, i.e. for 34.3% and 63.2% of the ³H and ¹⁴C, respectively, in the crude sterol fraction.²

**Analysis of Hydrocarbon Fraction**—Fig. 1 illustrates that the hydrocarbon fraction obtained from an incubation made with [2-¹⁴C]mevalonate, or with [1-¹⁴C]isopentenyl pyrophosphate, contains squalene as the sole radioactive component and also that in the gas-liquid radiochromatography instrument the ³H channel of the recording shows no change from the mean background counting level when a ¹⁴C-labelled fraction enters the counter. In contrast, examination by gas-liquid radiochromatography of the hydrocarbon fraction from the experiments recorded in Table I revealed three radioactive components (Fig. 2), a small component of ¹⁴C]squalene and two others containing both ³H and ¹⁴C. One of these doubly labelled components had a retention volume (Rₖ) of 1.26 and the other of 1.65 relative to that of squalene suggesting, in analogy with previous observations on homofarnesols (2), that they were homosqualene (1-methylsqualene) and bishomosqualene (1,24-dimethylsqualene) respectively.

The distribution of ³H and ¹⁴C in the components of the hydrocarbon fractions, based on gas-liquid radiochromatography analysis, is shown in Table II. Although the relative amounts of radioactivity in the three components differed in the two experiments (different enzyme preparation was used in each), the

### Table I

<table>
<thead>
<tr>
<th>Isotopes</th>
<th>Hydrocarbons</th>
<th>Crude sterols</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>³H</td>
<td>¹⁴C</td>
</tr>
<tr>
<td>Experiment 1</td>
<td>107,120</td>
<td>48,260</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>476,847</td>
<td>185,106</td>
</tr>
</tbody>
</table>

¹ In Experiment 2 the sterols were precipitated with digitonin; the digitonides contained 40,172 dpm of ³H and 47,874 dpm of ¹⁴C.

² The retention volumes (Rₖ) of radioactive components seen in gas-liquid radiochromatography, which had no corresponding mass detector responses on account of the low sensitivity of the mass detector, were taken from the midpoints of the integral curves of radioactivity.
The assumption that the two doubly labelled fractions were homo- and bishomosqualene was proved by mass spectrometric analysis of the hydrocarbon fraction from Experiment 2. The specimen left over after the gas-liquid radiochromatography analysis was introduced into the combined gas chromatography-mass spectrometry instrument which was fitted with the same gas chromatography column as the one used for gas-liquid radiochromatography. The specimen contained four unsaturated hydrocarbon fractions of a homologous series (n-C18H36, n-C20H40, n-C22H44, n-C24H48) emerging from the column before squalene, which could be separated into homo- and bishomosqualene by means of a 5% Dexsil column of 10% thickness. The radioactivity trace was recorded over a period of 10 minutes. The radioactivity distribution of the labelled fractions was calculated from data of gas-liquid radiochromatography.

The maximum slopes of the radioactivity tracings, at the half-value of the ascending limb of the gas chromatographic peak, do not correspond to the peaks of gas chromatographic records because the three pens of the threecolumn recorder are staggered one behind the other so that the two pens recording radioactivities are 5 and 7.5 mm, respectively, behind the pen writing the response of the mass detector. The discrepancy is further exaggerated on occasions when the gas chromatographic column is overloaded in respect of some component, as in Fig. 1 (slight overloading with squalene) and in Fig. 4 (Peak 1), resulting in a skewed non-Gaussian gas chromatographic peak approaching the shape of a rectangular triangle with a nearly perpendicular descending limb. In the latter instance the midpoint of the integral tracing of radioactivity is near the half-value of the ascending limb of the gas chromatographic peak.

Contamination of squalene specimens made from liver, or liver homogenates, by other hydrocarbons has been noted by one of us over many years. The origin of these hydrocarbons is unknown, but a dietary source is suspected. The substances found here were not of a random series of odd- and even-numbered n-paraffins, but only of the four monoenes; they were not radioactive.

The 3H:14C ratios in the equivalent doubly labelled fractions were very similar.

The 3H:14C ratios were derived by converting the radioactive counts to disintegrations per min by the use of counting efficiencies determined with 3H- and 14C-labelled toluenes. The sum of the percentages for each isotope in Experiment 1 does not add up to 100 because the specimen contained a small unidentified doubly labeled component, which emerged at some places by white paint around them.

The column used was the same as the one employed in the analysis of squalene shown in Fig. 1 except that it was run at 255°. The records of the 3H and 14C channels overlapped during the emergence of the first radioactive fraction after squalene. The mean counting rates have been drawn over the original record and these have been accentuated at some places by white paint around them.

The mean values of the 3H and 14C counts per minute obtained from a large number of gas chromatographic runs have been recorded in Table I. The amounts of 3H and 14C in each of the three labelled hydrocarbon fractions were calculated from the activities of the corresponding labelled toluenes, which were determined at the same time as the analysis of the hydrocarbon fraction from Experiment 2. The percentage distribution of 3H and 14C among components of hydrocarbon fractions calculated from data of gas-liquid radiochromatography is shown in Table II.
TABLE III

Percentage of distribution of H and 14C among components of sterol fractions as calculated from gas-liquid radiochromatographic analyses

<table>
<thead>
<tr>
<th>Chromatographic fraction</th>
<th>Experiment 1</th>
<th></th>
<th></th>
<th>Experiment 2</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H</td>
<td>14C</td>
<td>H:14C</td>
<td>H</td>
<td>14C</td>
<td>H:14C</td>
</tr>
<tr>
<td>Solvent front.</td>
<td>44.7</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Cholesterol, R, 1.0</td>
<td>0.0</td>
<td>35.6</td>
<td>0.0</td>
<td>0.0</td>
<td>47.80</td>
<td>0.0</td>
</tr>
<tr>
<td>Desmosterol, R, 1.14</td>
<td>29.0</td>
<td>22.2</td>
<td>3.14</td>
<td>0.0</td>
<td>11.60</td>
<td>0.0</td>
</tr>
<tr>
<td>Fraction, R, 1.26</td>
<td>29.0</td>
<td>22.2</td>
<td>3.14</td>
<td>0.0</td>
<td>11.60</td>
<td>0.0</td>
</tr>
<tr>
<td>Lanosterol, R, 1.43</td>
<td>0.0</td>
<td>22.2</td>
<td>0.0</td>
<td>0.0</td>
<td>13.05</td>
<td>3.76</td>
</tr>
<tr>
<td>Fraction, R, 1.73</td>
<td>96.3</td>
<td>37.6</td>
<td>3.17</td>
<td>31.4</td>
<td>8.70</td>
<td>3.73</td>
</tr>
</tbody>
</table>

- Data from the analysis of the crude sterol fraction (acetone-ether eluate from alumina column; cf. "Methods and Materials").
- Data from the analysis of digitonin precipitable sterols only.
- The basis of the calculations was the same as described in the footnote to Table II.

Fig. 3. Mass spectra of squalene (A); of the substance with a R, of 1.26 relative to that of squalene (B); and of the substance with a R, of 1.65 relative to that of squalene (C).
not aH, was associated with the gas chromatographic peaks of rigorous identification either by mass spectrometry or by chemical means. The amounts biosynthesized up to now were insufficient for labelled components are sterols rests at present on their precipitation with digitonin. Their retention volumes are those expected from the MeSi ethers of a methylcholesterol ("homocholesterol") and a methyllanosterol ("homolanosterol"). However, the amounts biosynthesized up to now were insufficient for rigorous identification either by mass spectrometry or by chemical means.

In addition to the two doubly labelled components, 14C, but not aH, was associated with the gas chromatographic peaks of cholesterol and lanosterol and also, in Experiment 2 with desmosterol.

**DISCUSSION**

The synthesis of squalene, homosqualene and bishomosqualene in incubations containing trans-[1-3H]3-methylpent-2-enyl pyrophosphate and [1-14C]isopentenyl pyrophosphate indicates that Reactions 1, 2, and 3, described in the introduction, have occurred in the enzyme preparations and also that the squalene synthetase system of liver microsomes condensed readily not only farnesyl pyrophosphate with homofarnesyl pyrophosphate, but also the latter with itself.

The theoretical ratio of aH to 14C in homofarnesyl pyrophosphate synthesized according to Reaction 3 should be 6/2 × 0.47 = 6.38 and the same value should also apply to bishomosqualene (2 × 6/4 × 0.47). The found values of 6.93 and 6.41 (Table II) are reasonably close to these predicted values considering the errors of reading the levels of radioactive counts from the gas-liquid radiochromatography records and converting these into disintegrations.

Since homosqualene, synthesized by the condensation of [9-3H,1,5-14C]12-methylfarnesyl pyrophosphate with [1,5,9,14C]farnesyl pyrophosphate (cf. Reactions 2 and 3), should contain only one aH-labelled position and five positions labelled with 14C, the aH:14C ratio in this substance should be 6/5 × 0.47 = 2.55. The values found, 3.10 and 3.66 (Table II), particularly in Experiment 2, were substantially higher than predicted. Some systematic error of counting might be the explanation for the anomalous data. An alternative explanation might be that unlabelled farnesyl pyrophosphate present in the undialyzed enzyme preparations diluted the [14C]farnesyl pyrophosphate. We have no certain information to choose between these alternatives.

FIG. 4. Copy of gas-liquid radiochromatographic record obtained from the analysis of steroids, as the MeSiO derivatives, recovered from the digitonides of Experiment 2 (cf. Table I and text). Column: 10% Dexsil, 6 feet, 285°. Mass detector (thermal conductivity cell) response at top; Peak 1, cholesterol; Peak 2, desmosterol; Peak 3, 24,25-dihydrolanosterol; Peak 4, lanosterol.1

The evidence for the two doubly labelled components found in the sterol fractions to be truly sterols (homocholesterol and homolanosterol) rests at present only on their being precipitable by digitonin and on their having retention volumes in gas-liquid chromatography commensurate with those expected of sterols. Although we need more conclusive proof, we believe the data give strong indication of the cyclization of homosqualene, but not of bishomosqualene, to a sterol. We found no evidence by the gas-liquid radiochromatography analyses of the sterol fractions from either experiment to suggest a component derived from the cyclization of bishomosqualene, i.e. a component having a aH:14C ratio similar to that of bishomosqualene.

Oxidation of squalene to the 2,3-epoxide is known to precede cyclization to sterol (10–13). Homosqualene might be epoxidized at either end of the molecule (Scheme I), but each oxide form (II and V) would lead to a different type of homolanosterol: homolanosterol III would contain the 3H-labelled position in ring IV and the 4-gemdimethyl group of lanosterol would be replaced by the 4-ethyl-4-methyl groups; homolanosterol VI would contain the aH-label in the side chain and the extra methyl group at C-27. The transformation of 27-methyllanosterol (VI) to 27-methylcholesterol (and intermediates) could proceed probably without difficulty. If homolanosterol III has been formed and transformed into cholesterol the latter should have contained aH; we found only 14C associated with cholesterol.

The fact that we did not find a component in the sterol fractions that might have been derived from the cyclization of bishomosqualene suggests that the isobutylidene structures at each end of bishomosqualene prevented enzymic epoxidation and, hence, cyclization. The apparent cyclization of homosqualene, but not of bishomosqualene, to a sterol suggests that homosqualene may be epoxidized only at its isopropylidene end since the
only difference between bishomosqualene and homosqualene is that the former contains the isobutylidene group at both ends whereas the latter contains at one end an isobutylidene and at the other an isopropylidene group. We exclude, therefore, the reaction sequence I \( \rightarrow \) III in Scheme 1 and suggest that the doubly labelled substances found in the sterol fractions of the experiments were derived by the reaction sequence IV \( \rightarrow \) VI (Scheme 1) and its sequelae.

Corey et al. (14) and Crosby et al. (15), in their studies on the enzymic cyclization of homologs of squalene 2,3-oxide, observed the formation of a homolanosterol in incubations of 2,3-oxidosqualene stereol cyclase with a racemic mixture of chemically synthesized cis- and trans-epoxides of 1-methylalquolene. Crosby et al. (15) concluded that only that isomer of the squalene-epoxide homolog in which the unaltered terminal methyl group was cis to the main chain, corresponding to Structure 11 in Scheme 1 and expected to be synthesized from trans-3-methylpent-2-enyl-pyro-phosphate, was cyclized since the homolanosterol formed was identified as the 4α-ethyl-14β, 14a-dimethylcholesta-8,24-dien-3β-ol. Thus if the bishomosqualene synthesized in our experiments had been epoxidized it should have been cyclized enzymically. The findings of Crosby et al. (15), taken in conjunction with our own, reinforce our conclusion that the isobutylidene end of homosqualene cannot be epoxidized enzymically and support our tentative assignment of the structure of the homosterols as 27-methyl compounds. This conclusion is also supported by the relative retention volumes of the homosterols in gas chromatography. If the extra methyl group had been attached to the nucleus of the sterols, the increase in the retention volumes should have been much less than observed. It is generally true that in a homologous open chain structure the addition of a methylene group in the chain increases the retention volume by a factor of 1.2 to 1.3; the effects of additions of branched methyl groups onto a ring system are less pronounced. The data of Crosby et al. (15) indicate, e.g. that the retention volume of the MS/MS ether of 4α-ethyl-14β, 14a-dimethylcholesta-8,24-dien-3β-ol relative to that of lanosterol was only 1.05.

A “unidirectional” cyclization of the asymmetrically synthesized squalene (16) to lanosterol has been explored before (17, 18) with inconclusive results. Our data suggest for the first time that a minor chemical modification of the squalene structure may induce the enzymic cyclization to be initiated from one specific end of the molecule.

We have also examined our data as to whether they might suggest differences in the reactivities of farnesyl pyrophosphate and of homofarnesyl pyrophosphate in the squalene synthetase system. To this end we have calculated from the amount of the various products observed in Experiment 2 the amounts of farnesyl pyrophosphate and of homofarnesyl pyrophosphate that must have been synthesized in the enzyme preparation. The calculations were based on the 14C content of each component and on the specific activity of the 14C-isopentenyl pyrophosphate used, 1.043 dpm per nmole. Thus, e.g. 22,986 dpm of 14C in cholesterol was taken to be equivalent to 22,986/1043 = 21.85 nmole of isopentenyl residues and to 21.85/6 = 3.68 nmole of cholesterol, which needed the same molar amount of squalene pyrophosphate as cholesterol.

1 The structural assignment was made by nuclear magnetic resonance spectrometry. However the authors refer to the same compound also as the “4α-ethyl sterol” (cf. second paragraph, right-hand column, p. 533 in Reference 15). We are assuming that the 4α-ethyl sterol was meant throughout.

<table>
<thead>
<tr>
<th>Product</th>
<th>Isopentenyl residues converted</th>
<th>Product synthesized</th>
<th>Farnesyl pyrophosphate equivalents</th>
<th>Homofarnesyl pyrophosphate equivalents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squalene</td>
<td>9.29</td>
<td>1.55</td>
<td>3.10</td>
<td>0.0</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>21.95</td>
<td>3.66</td>
<td>7.32</td>
<td>0.0</td>
</tr>
<tr>
<td>Desmosterol</td>
<td>5.32</td>
<td>0.89</td>
<td>1.78</td>
<td>0.0</td>
</tr>
<tr>
<td>Lanosterol</td>
<td>5.99</td>
<td>1.00</td>
<td>2.00</td>
<td>0.0</td>
</tr>
<tr>
<td>Homosqualene</td>
<td>53.66</td>
<td>6.73</td>
<td>6.73</td>
<td>6.73</td>
</tr>
<tr>
<td>“Homocholesterol&quot;</td>
<td>8.65</td>
<td>1.73</td>
<td>1.73</td>
<td>1.73</td>
</tr>
<tr>
<td>“Homolanosterol&quot;</td>
<td>3.99</td>
<td>0.80</td>
<td>0.80</td>
<td>0.80</td>
</tr>
<tr>
<td>Bishomosqualene.</td>
<td>19.73</td>
<td>4.93</td>
<td>9.86</td>
<td>9.86</td>
</tr>
<tr>
<td>Total</td>
<td>108.58</td>
<td>21.29</td>
<td>23.40</td>
<td>19.12</td>
</tr>
</tbody>
</table>

* The 14C content of the hydrocarbon and crude sterol fraction was equivalent to the utilization of 155.3 nmole of isopentenyl pyrophosphate. The lower recovery recorded here is accounted for by the loss of radioactivity in the supernatant after precipitation of sterols with digitonin (cf. text and Table I).

<table>
<thead>
<tr>
<th>Products</th>
<th>% Distribution</th>
<th>Calculated</th>
<th>Observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squalene + sterols</td>
<td></td>
<td>30.47</td>
<td>33.4</td>
</tr>
<tr>
<td>Homosqualene + homosterols</td>
<td></td>
<td>49.40</td>
<td>48.4</td>
</tr>
<tr>
<td>Bishomosqualene.</td>
<td></td>
<td>20.07</td>
<td>23.2</td>
</tr>
</tbody>
</table>

* A random association of farnesyl and homofarnesyl pyrophosphate was assumed from a mixture consisting of 55.2% of the former and 44.8% of the latter substrate.

as precursor and which, in turn, required the availability of 3.66 × 2 = 7.32 nmole of farnesyl pyrophosphate for synthesis. Homosqualene and the two “homosterols” were assumed to contain 5 and bishomosqualene was assumed to contain 4 isopentenyl residues per molecule. The calculations gave the data of Table IV, which show that 23.40 nmole of farnesyl pyrophosphate and 19.12 nmole of homofarnesyl pyrophosphate had to be synthesized first in order to account for the various products. The calculated and observed distributions of products synthesized from such a mixture of farnesyl and homofarnesyl pyrophosphate are shown in Table V. The data suggest an almost random combination of farnesyl pyrophosphate with homofarnesyl pyrophosphate. In contrast, the conversion of homosqualene to homosterols, if the doubly labelled components found in the sterol fractions were indeed sterols, was very uneven (cf. Table IV). In the homosqualene plus homosterol fractions of Experiment 2 only 2.53 nmole were identifiable as sterols out of 9.96 nmole of total products, whereas 5.55 nmole were found in sterols and only 1.55 nmole in squalene in the singly 14C-labelled squalene plus sterol components. This difference is also well illustrated by the finding of only small amounts of newly syn-
thesized squalene left in the hydrocarbon fractions which were dominated by homo- and bishomosqualene (cf. Fig. 2). If homosqualene cannot be epoxidized at its isobutylidene end, as we have inferred, its rate of conversion to sterols should be much slower than that of squalene.

Acknowledgments—We thank Mr. Jean Bardenheier and Dr. Cheng Liao for their assistance with the preparation of the \textit{trans}-[1-\textsuperscript{3}H]\textit{J}-methylpent-2-enyl pyrophosphate.

REFERENCES
