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ENZYMATIC SYNTHESIS OF BISHOMOFARNESYL PYROPHOSPHATE WITH THE INSECT JUVENILE HORMONE SKELETON

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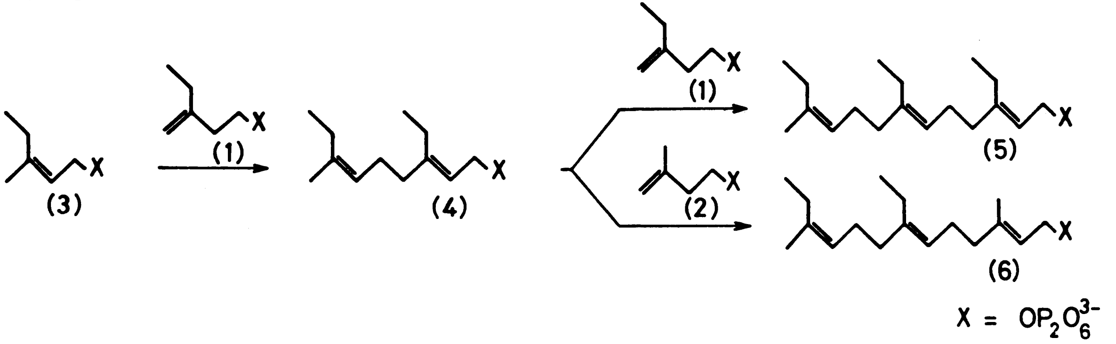
trans,trans,cis-3,11-Dimethyl-7-ethyltrideca-2,6,10-trien-1-ol and its pyrophosphate whose carbon skeletons were the same as that of insect juvenile hormone were synthesized from cis-3-methylpent-2-enyl pyrophosphate, 3-ethylbut-3-enyl pyrophosphate and isopentenyl pyrophosphate by the action of farnesyl pyrophosphate synthetase.

We have been studied the substrate specificity of farnesyl pyrophosphate synthetase and reported recently that only a homoisopentenyl pyrophosphate, 3-ethylbut-3-enyl pyrophosphate (1) among 3-alkylbut-3-enyl pyrophosphates could act as a substrate for liver farnesyl pyrophosphate synthetase to condense with dimethylallyl pyrophosphate or geranyl pyrophosphate in place of isopentenyl pyrophosphate (2)1). This finding, combined with the earlier observation that the farnesyl pyrophosphate synthetase has a relatively low specificity with regard to the allylic substrate2-7), led us interested in the enzymatic synthesis of bishomofarnesyl pyrophosphate having the same carbon skeleton as that of insect juvenile hormone.

In the first place we examined the condensation between the two artificial substrates, 1 and cis-3-methylpent-2-enyl pyrophosphate (3) by the action of farnesyl pyrophosphate synthetase. The incubation mixture for the enzymatic reaction contained, in a final volume of 4.0 ml, 80 μmol of Tris-HCl buffer, pH 7.7, 20 μmol of $MgCl_2$, 200 nmol of 3, 100 nmol of $[1\text{-}^3H]$-1 (0.45 μCi), 20 μmol of potassium fluoride and ca. 0.4 mg of farnesyl pyrophosphate synthetase purified from pig liver8). After the incubation at 37° for 30 min, the reaction mixture was treated with alkaline phosphatase as usual3-7), and the radioactive materials extracted with petroleum were subjected to radio-g.l.c. and t.l.c. The radio-g.l.c. of the materials showed two peaks of radioactivity at 8.0 (A) and at 21.0 min (B) with nearly equal intensity under the condition where geraniol and trans,trans-farnesol emerged at 4.8 and 16.0 min, respectively (Fig. 1-a). These two radioactive materials developed at R_f 0.55 (A) and 0.65 (B) on t.l.c. in a system of benzene - ethyl acetate (4 : 1), in which the R_f for geraniol and trans,trans-farnesol were 0.49 and 0.53, respectively. In order to identify these products, they were extracted from the t.l.c. plate and subjected to g.l.c.-mass spectrometry. The mass spectrum for A showed peaks at m/e 182 (M^+) corresponding to $C_{12}H_{22}O$, 164, 151, 135, 107, 83 and 55 which was the base peak, supporting the structure of bishomogeraniol (4' : X = OH in 4). The mass spectrum for B was compatible with the structure of trishomofarnesol (5' : X = OH in 5). The molecular ion was found at m/e 264 ($C_{18}H_{32}O$) with fragments at 246, 233, 163, 135, 107, 95 and strong peaks at 83 and 55. The strong peaks at m/e 83 and 55 observed in both A and

B are attributable to homologous fragments (C_6H_{11} and C_4H_7) corresponding to those at m/e 69 (C_5H_9) and 41 (C_3H_5) observed as characteristic peaks for normal prenols such as geraniol and farnesol. For a further confirmation authentic samples of *cis,cis*- and *trans,cis*-bishomogeraniol (4') were prepared according to the method of Dahm et al.9), and the radioactive alcohol A derived enzymatically from 1 and 3 was identified with the authentic specimen of *trans,cis* isomer 4' by t.l.c., g.l.c. and mass spectrometry. The alcohol B derived from 1 and 3 was also identified with the alcohol resulting from the enzymatic condensation of 1 with *trans,cis*-bishomogeranyl pyrophosphate (4) which was synthesized by the chemical phosphorylation of the authentic alcohol 4' (Fig. 1-b). Hence, it was verified that 4 was accumulated as an intermediate during the formation of trishomofarnesyl pyrophosphate (5) from 1 and 3 catalyzed by farnesyl pyrophosphate synthetase.



These results suggest a possibility of the enzymatic condensation of this bishomogeranyl pyrophosphate (4) with isopentenyl pyrophosphate (2) to give bishomofarnesyl pyrophosphate (6) with the carbon skeleton of insect juvenile hormone. Thus, 4 and [1-^{14}C]-2 were incubated with the enzyme under a condition similar to that described above, and the product was similarly analyzed. The radio-g.l.c. in the same condition as described above showed a single radioactivity peak (C) at a retention time of 20.1 min which is reasonable for bishomofarnesol (Fig. 1-c). The g.l.c.-mass spectrum of this material showed a molecular ion at m/e 250 ($C_{17}H_{30}O$) with fragments at 232, 219, 175, 135, 107, 95, 83 and 55 which was the base peak. These results support the bishomofarnesol (6' : X = OH in 6) structure. The yield of 6 was *ca.* 20 % based on [1-^{14}C]-2.

These facts led us try to synthesize 6 directly from 1, 2 and 3 by the action of farnesyl pyrophosphate synthetase. After [1-^{3}H]-1 and 3 were incubated with the enzyme for 30 min in the same condition as described above, 50 nmol of non-labeled 2 was added to the mixture and incubated for another 4.5 hr. After the enzymatic hydrolysis the petroleum extracts were subjected to radio-g.l.c. The radioactive materials originated from [1-^{3}H]-1 emerged at retention times of 20.1 and 21.0 min (Fig. 1-d). The former was coincided to that of the bishomofarnesol 6' synthesized from 2 and 4, and the latter was found to be trishomofarnesol 5'. The ratio of formation of 5 and 6 was *ca.* 1 : 1. The yield of the formation of 6 was *ca.* 5 % on the basis of [1-^{3}H]-1. Formation of [^{3}H,^{14}C]-labeled 6 and [^{3}H]-labeled 5 was also observed when [1-^{3}H]-1, [1-^{14}C]-2 and non-labeled 3 were incubated at the same time, though the main product was [^{14}C]-labeled homofarnesyl pyrophosphate, *trans,trans,cis*-3,7,11-trimethyltrideca-2,6,10-trienyl pyrophosphate2,3).

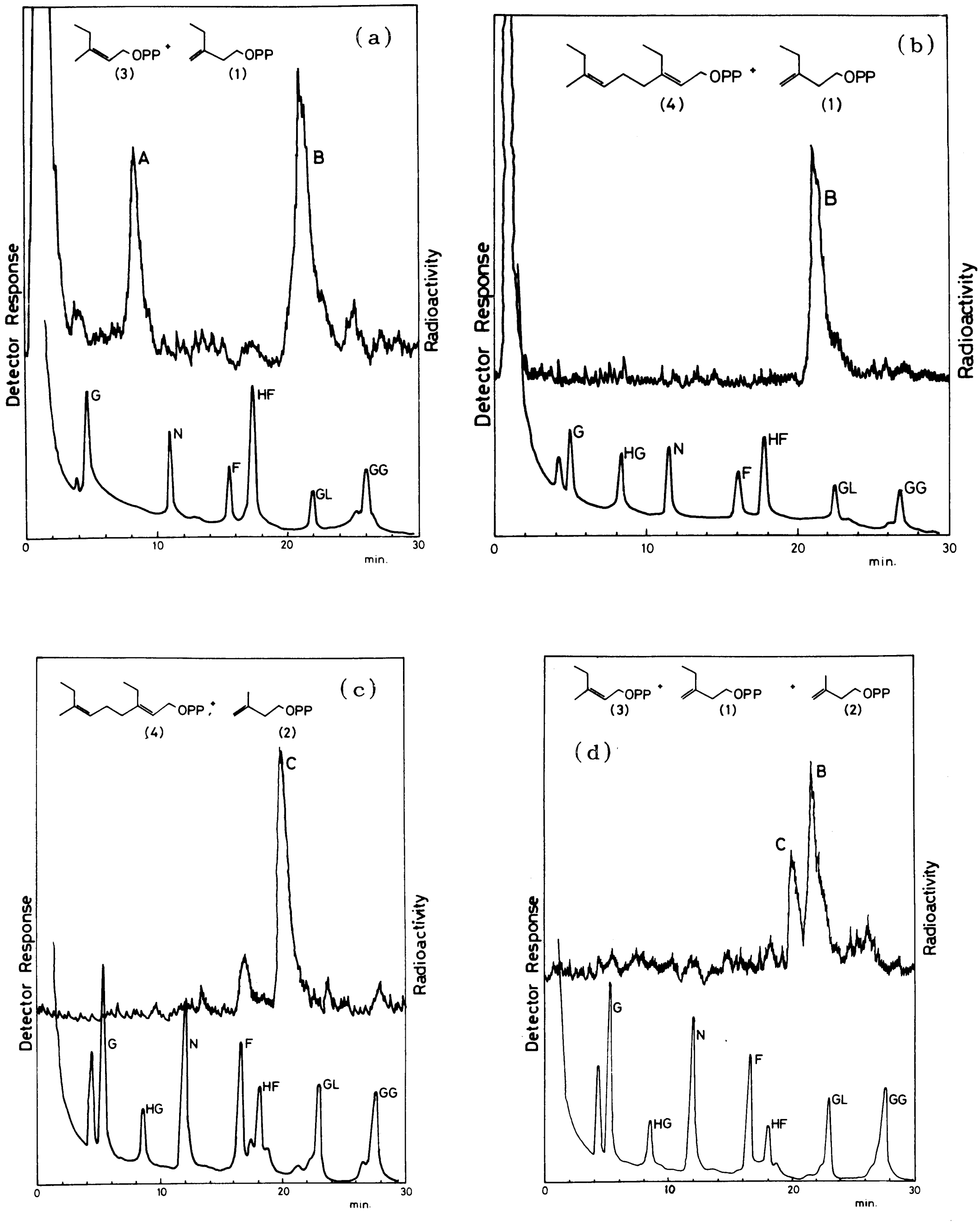


Fig. 1. Radiogas-chromatograms of alcohols derived from [1-^{3}H]-1 and 3 (a); [1-^{3}H]-1 and 4 (b); [1-^{14}C]-2 and 4 (c); and [1-^{3}H]-1, 2 and 3 (d).

The chromatography was carried out at a linear programmed temperature at a rate of 4°/min from 100° to 200° on a 1 m column of 1.5 % OV-17.
Reference alcohols: G, geraniol; HG, 4'; N, trans-nerolidol; F, trans,trans-farnesol; HF, trans-homofarnesol$^{1)}$; GL, trans,trans-geranyllinalool; GG, all-trans-geranylgeraniol.

Thus, the same carbon skeleton as that for the insect juvenile hormone was framed from C_5 and C_6 compounds by the action of farnesyl pyrophosphate synthetase of pig liver. The same enzyme is known to be present widely in various organisms, and it is of interest to know whether 3-ethylbut-3-enyl pyrophosphate (1) is actually involved in the biosynthesis of insect juvenile hormone.

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