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The Biosynthesis of Linalool in *Cinnamomum Camphora* Sieb. *var. linalooliferum* Fujita[1)]

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The labelling pattern in linalool biosynthesized from [2-^{14}C] mevalonic acid in *Cinnamomum Camphora* Sieb. *var. linalooliferum* Fujita was found to be consistent with the predicted biosynthetic pathway involving the condensation of isopentenyl pyrophosphate with 3,3-dimethylallyl pyrophosphate. The radioactivities at C-4 and C-8 (and/or C-10) were about equal, in contrast to the asymmetric pattern of labelling observed for bicyclic monoterpenes, which contained a tracer predominantly in one position of the skeleton derived from isopentenyl pyrophosphate. The efficiency of the incorporation of the tracer into linalool was almost independent of the season of the examination, although a slight increase was observed in the season of the active growth of the plant.

The biosynthesis of monoterpenes from mevalonic acid (MVA) involves the condensation of isopentenyl pyrophosphate (IPP) (I) with 3,3-dimethylallyl pyrophosphate (DMAPP) (II) directed towards the formation of geranyl pyrophosphate (GPP) (III) or neryl pyrophosphate, the presumed precursors of acyclic and cyclic monoterpenes respectively.[2)] Recently, thujane derivatives and camphor biosynthesized from [2-^{14}C] MVA were shown[3,4)] to contain their tracer to a great extent at one ring position derived from such a tracer-containing precursor as IPP (I), but not DMAPP (II). Such examples of preferential labelling being detected for one of the participating C_5-units have also been reported on sesquiterpenes, coriamyrtin and tutin,[5)] though other investigators[6)] have observed an equal distribution of labelling in tutin. In order to clarify the generality of these unexpected results, we have tested the labelling pattern and its seasonal variation in linalool biosynthesized from [2-^{14}C] MVA in *Cinnamomum Camphora* Sieb. *var. linalooliferum* Fujita (Hōsho in Japanese). In the biosynthesis of linalool, which is an acyclic monoterpene probably derived from GPP (III) in the initial stages, the situation was different from those observed for the bicyclic monoterpenes.[3,4)]

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Results

Efficiency of the Incorporation of a Tracer. In order to test the seasonal variation of the incorporation of the tracer, [2-^{14}C] MVA was applied by stem-feeding into the leaves at three different seasons, April, July, and November. The results of the incorporation of the tracer into linalool are shown in Table 1.

TABLE 1. SEASONAL VARIATIONS OF ESSENTIAL OIL AND LINALOOL AND OF THE INCORPORATION OF [2-^{14}C] MVA INTO LINALOOL

	April	July	November
Essential oil (w/w%)[a)]	0.48	1.20	1.64
Linalool (w/w%)[a)]	0.27	0.60	0.85
Incorporation (%)	0.013	0.022	0.012

a) Of the plant

Labelling Patterns. The selective hydrogenation of linalool (IV) on platinum oxide afforded 1,2-dihydrolinalool, which was degraded to acetone containing a tracer from C-7, C-8, and C-10, and to 4-methyl-4-hexanolide (V) containing a tracer from C-1 to C-6 and

C-9, by permanganate-periodate oxidation.[7,8] Acetone was degraded to iodoform containing a tracer from C-8 and C-10 and to acetic acid. Acetic acid thus produced was further cleaved to methylamine and to carbon dioxide containing a tracer originating from C-7. The lactone (V) was subjected to Kuhn-Roth oxidation[9] to obtain acetic acid containing a tracer from C-1 to C-3 and C-9. Linalool and all its degradation products were converted into barium carbonate by Van Slyke-Folch oxidation[10] in order to determine their specific activities. The results are shown in Table 2.

Table 2. Specific radioactivities of linalool and its degradation products

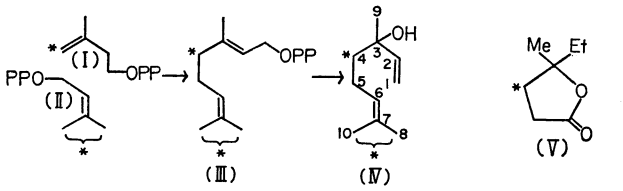
Compounds (Carbons originated from IV)	Specific radioactivity, dpm/mM (%)		
	April	July	November
Linalool (C-1—C-10)	5717	6169	607
Iodoform (C-8 and/or C-10)	1025(17.9)	1335(21.6)	124(20.4)
Acetic acid (C-7, 8, and /or C-7, 10)	1206(21.1)	1524(24.7)	154(25.3)
Methylamine (C-8 and/or C-10)	972(17.0)		
Carbon dioxide (C-7)	114(2.0)		
4-Methyl-4-hexanolide (C-1—C-6, and C-9)	3167(55.4)	3274(53.1)	331(54.4)
Acetic acid (C-1—C-3, and C-9)	363(6.3)	265(4.3)	26(4.2)

Seasonal Variation in Labelling Patterns. The labelling patterns of linalool obtained from the feeding experiments were determined at three different seasons of growth; the results are shown in Table 2.

Discussion

Both the yield of the essential oil and the content of linalool increased with the growth of the plant, as is shown in Table 1. A similar trend has previously been reported[11] in the same plant. Though a slight increase in the incorporation of the tracer into linalool was observed in July, which is the actively-growing season of *C. Camphora*, the efficiency of incorporation was judged to be almost independent of the season, as the increment was too small to discuss any seasonal dependence. The incorporations are, although low, similar to the values found in analogous experiments.[3,4,12]

The extensive degradation of linalool, obtained from feeding in July, revealed the location of 46% of the



* indicates ^{14}C; OPP indicates a pyrophosphate group.

incorporated tracer on acetone and that of the residual activities (54%) on the lactone (V). Acetic acid derived from the lactone (V) showed, unfavorably, a small activity. This is presumably attributable to the incorporation of the C_1-pool which resulted from the degradation of a small amount of the applied MVA.[13,14] Thus, the main site of labelling on the lactone (V) was, on biogenetic grounds, considered to be the position which corresponds to the C-4 of linalool (IV). With respect to the pattern of labelling, the results obtained above have made us conclude as follows: (a) The pattern of labelling observed for linalool is consistent with the biosynthetic mechanism shown in the Scheme. (b) The specific activities at C-4 and C-8 (and/or C-10), originating from IPP (I) and DMAPP (II) respectively, are nearly equal. The almost balanced pattern of labelling in linalool, an alicyclic monoterpene, is in contrast with the asymmetric pattern of labelling observed for some cyclic monoterpenes.[3,4] The equality of the labelling means that the skeleton results from the condensation of IPP (I) with DMAPP (II), both of which have the same specific activities. It thus appears that the formation of DMAPP (II) in the plant under these conditions is caused by the isomerization of the radioactive IPP (I) generated from the applied [2-^{14}C] MVA. (c) The methyl group and the terminal methylene of IPP (I) are non-equivalent, in the same manner as they are in the biosynthesis of other mono- and higher-terpenes.[15,16]

It has been supposed[3] that the season affects the amount of enzymes, which in turn cause the asymmetrical labelling in thujone. We have now examined the seasonal effect on the radioactivities at C-4 and C-8 of linalool biosynthesized in *C. Camphora*. The specific radioactivities in July and November were comparable for the two carbon atoms (Table 2), but they were slightly unbalanced in April, the sprouting season of this plant. These facts seem to indicate that the active enzyme catalyzing the conversion of IPP (I) to DMAPP (II) may not be sufficient in the sprouting season. Thus, the condensation of the labelled IPP (I) with the unlabelled DMAPP (II) derived from the metabolic pool would lead to the low radioactivities in the part of the skeleton originating from DMAPP (II). However, the unbalance of radioactivities in April was very slight as compared with that in the cases of thujane derivatives[3] and camphor.[4] Although it has been suggested[3,4] that the asymmetrical labelling may be general for the biosynthesis of monoterpenes in leaves of higher plants, the

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radioactivities derived from [2-^{14}C] MVA, on the contrary, were found to be almost equally distributed between the C-4 and C-8 of linalool, an alicyclic monoterpene.

Experimental

Materials. Specimens of *C. Camphora* were grown from young trees obtained from the experimental farm of the Soda Perfumery Co., Kagoshima Prefecture, Japan. Feeding experiments were carried out on small terminal branches (*ca.* 15 cm long) of three-year-old plants, from April to November. *C. Camphora* sprouts from March to April; the growth is more active in July than in November.

Administration of the Labelled Compound. A phosphate buffered solution (40 m*l*, pH 7.38) of [2-^{14}C] MVA (0.1 mCi, 17 μM), and ATP (0.1 mM) was fed through a cut-stem into small twigs (*ca.* 150 g). Immediately before use, the MVA lactone was converted into the potassium salt of MVA by a potassium hydroxide solution. After the solution had been fed in, the foliage was maintained in a phosphate-buffered solution (pH 7.38) for one day before harvesting.

Isolation of Radioactive Linalool. The harvested leaves and stems were subjected to steam distillation. The oil thus obtained (0.71 g) was subjected to column chromatography on silica gel (less than 0.08 mm, Merck). Elution by a mixture of *n*-hexane with increasing amounts of ethyl acetate afforded radioactive linalool (0.40 g), which was judged to be pure by analytical tlc and glc.

Degradation of Linalool. Linalool (598 mg) in methanol (3 m*l*) was selectively hydrogenated on Adams platinum oxide (4 mg) to 1,2-dihydrolinalool (600 mg). The dihydrolinalool (200 mg) was oxidized with a permanganate-periodate reagent[7,8] for 24 hr. After the excess oxidant had been reduced with a minimum of sodium bisulfite, the weakly alkaline solution was steam-distilled. The ether extraction of the acidified residual solution afforded 4-methyl-4-hexanolide (V) (98 mg; its *S*-benzylthiouronium salt, mp 132—133°C, lit,[17] mp 132—132.5°C). The aqueous distillate was subjected to hypoiodite oxidation. Iodoform (369 mg) filtered off was purified by sublimation at reduced pressure. The filtrate was acidified with sulfuric acid and treated with silver sulfate to remove iodine. The mixture was then steam-distilled. The evaporation of the neutralized distillate afforded acetic acid as the sodium salt (38 mg). The salt was purified by means of the addition of dry ether to its dry ethanol solution. The Schmidt reaction[18] of the purified salt afforded carbon dioxide and methylamine, which were then converted to barium carbonate and its hydrochloride respectively. The Kuhn-Roth oxidation[9] of 4-methyl-4-hexanolide (V) (71 mg) gave acetic acid as the sodium salt (94 mg); this was then refined as above.

Radioassay. Linalool and all its degradation products were converted to barium carbonate by Van Slyke-Folch oxidation, and aliquots of barium carbonate were counted on planchets at an infinite thickness under a Aloka TDC R 1361 2π-gas-flow detector. The counting error for the values shown in Tables 1 and 2 is about $\pm 3\%$.

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