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The Biosynthesis of Monoterpenoids in Higher Plants. The Biosynthetic Pathway Leading to the Monoterpenoids from Amino Acids with a Carbon-skeleton Similar to Mevalonic Acid

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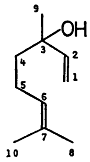
Radioisotopically labeled L-leucine, L-valine, DL-alanine, sodium acetate, and DL-mevalonic acid were incorporated into linalool by the intact plant of *Cinnamomum camphora* Sieb. var. *linalooliferum* Fujita and into geraniol and citronellol by that of *Pelargonium roseum* Bourbon. The uptake of leucine and valine resulted in the preferential location of the radioactivity on the 3,3-dimethylallyl pyrophosphate-derived moiety of these acyclic monoterpenoids, whereas the uptake of alanine resulted in the preferential location on the isopentenyl pyrophosphate-derived moiety, much as in the cases of mevalonic acid and sodium acetate. A biosynthetic pathway leading to the monoterpenoids from the amino acids is discussed.

The biosynthesis of monoterpenoids is believed to involve the conversion of mevalonic acid (MVA)[1] into isopentenyl pyrophosphate (IPP)[1] and 3,3-dimethylallyl pyrophosphate (DMAPP),[1] followed by the condensation of IPP with DMAPP directed toward the formation of monoterpenoids.[2] The predominant location of the radioactivity on the IPP-derived moiety has been observed for the biosynthesis of monoterpenoids from MVA-2-^{14}C by the leaves of higher plants,[3–6] in contrast to the equal distribution in the IPP- and DMAPP-derived moieties of squalene and triterpenoids biosynthesized from MVA-2-^{14}C by *Pisum sativum.*[7] The unbalanced distribution of the labeling in the monoterpenoids has been explained in terms of the operation of several factors, which involve the participation of some other pathway or intermediates in the DMAPP formation. No incorporation, or only a slight one, of leucine and valine into monoterpenoids by higher plants has been reported,[3,8,9] but the biosynthetic pathway of steroids from leucine *via* MVA has been established in animal tissues,[10–13] and it is highly probable that such a pathway takes part in higher plants, also.

We have performed the feeding experiments of radioisotopically labeled leucine, valine, and alanine into linalool (**1**) with the intact plant of *Cinnamomum camphora* Sieb. var. *linalooliferum* Fujita, and into geraniol (**2**) and citronellol (**3**) with that of *Pelargonium roseum* Bourbon, and then compared the distribution of the radioactivity originating from these precursors in the IPP- and DMAPP-derived moieties of the monoterpenoids with its distribution in the moieties of the monoterpenoids biosynthesized from ^{14}C-labeled MVA and acetate. The results have been partly outlined in the preliminary communications.[14–17] We here wish to report, *en bloc*, details of the results.



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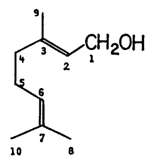

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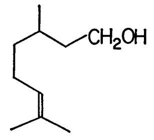

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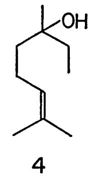

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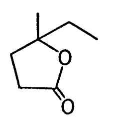

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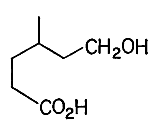

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Results

*The Incorporation of the Radioisotopically Labeled Precursors into Linalool (**1**), Geraniol (**2**), and Citronellol (**3**).* Feeding experiments were carried out on sprigs of *C. camphora* and *P. roseum.* The radioisotopically labeled precursors used for the incorporation were L-leucine-U-^{14}C,[18] L-valine-U-^{14}C, DL-alanine-2-^{14}C, sodium acetate-2-^{14}C, and DL-mevalonic-2-^{14}C acid. A phosphate-buffered solution (pH 7 4) of each precursor was fed through the cut stem to the leaves of the plants. The leaves and stems were subsequently steam-distilled to give an essential oil, which was then subjected to preparative TLC to isolate **1** from *C. camphora* and **2** and **3** from *P. roseum.* The radioactivities of **1**, **2**, and **3** were measured in Bray's scintillation solvent[19] with a liquid scintillation spectrometer; they are shown in Tables 1 and 2. Leucine and valine were incorporated into **1**, **2**, and **3** in the range of 0.0002—0.0055%. The incorporation of alanine into these monoterpenoids was at a considerably high level (0.014—0.076%), whereas that of acetate was at a low level (0.004—0.027%).

*The Distribution of Radioactivity in Linalool (**1**) Biosynthesized from ^{14}C-Labeled Leucine, Valine, and MVA by* C. camphora. Linalool (**1**), biosynthesized from leucine-U-^{14}C, valine-U-^{14}C, and MVA-2-^{14}C, was subjected to degradation to determine the labeling pattern. The radioactive linalool (**1**) isolated was hydrogenated in the presence of platinum dioxide to give 1,2-dihydrolinalool (**4**), which was subsequently degraded to 4-methyl-4-hexanolide (**5**) containing the C-1—C-6 and C-9 carbon atoms of **1**, and to acetone containing the C-7, C-8, and C-10 carbon atoms of **1**, by permanganate-periodate oxidation.[20] The hexanolide (**5**) and acetone were then converted to the *S*-benzylthiouronium salt[21] and the thiosemicarbazone

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Table 1. Incorporation of the radioisotopically labeled precursors into linalool (**1**) by *C. camphora*

Exptl No.	Precursors^{a)} (mCi)		Feeding time^{b)} h	Season	Sp. act.^{c)} dpm/mmol	Incorp. %
1	Leu-U-^{14}C	(0.02)	24	April	1.40×10^{2}	0.0010
2	Leu-U-^{14}C	(0.025)	24	May	1.70×10^{2}	0.0040
3	Val-U-^{14}C	(0.01)	24	July	7.52×10^{2}	0.0055
4	Val-U-^{14}C	(0.005)	12	Oct.	5.92×10^{2}	0.0015
5	Val-U-^{14}C	(0.02)	24	Oct.	2.72×10^{2}	0.0015
6	Ala-2-^{14}C	(0.05)	24	June	1.86×10^{4}	0.076
7	Ala-2-^{14}C	(0.02)	24	July	3.00×10^{3}	0.026
8	NaOAc-2-^{14}C	(0.12)	24	June	1.10×10^{4}	0.0043
9	NaOAc-2-^{14}C	(0.12)	24	July	3.76×10^{4}	0.0075
10	MVA-2-^{14}C	(0.10)	24	June	4.39×10^{3}	0.022^{d)}
11	MVA-2-^{14}C	(0.04)	24	July	3.15×10^{3}	0.086^{d)}

a) Leu-U-^{14}C, Val-U-^{14}C, Ala-2-^{14}C, NaOAc-2-^{14}C, and MVA-2-^{14}C denote L-Leucine-U-^{14}C, L-valine-U-^{14}C, DL-alanine-2-^{14}C, sodium acetate-2-^{14}C, and DL-mevalonic-2-^{14}C acid respectively. b) Metabolic period after uptake of tracer. c) Values cannot be compared among different batches of incorporations, since different quantities of the carrier and/or the tracer are used. Sp. act. denotes specific radioactivity. d) Calculated as the only (3*R*)-enantiomer of a racemic mixture of the substrate participates in the formation of **1**.

Table 2. Incorporation of the radioisotopically labeled precursors into geraniol (**2**) and citronellol (**3**) by *P. roseum*

Exptl No.	Precursors^{a)} (mCi)		Feeding time^{b)} h	Season	Geraniol (**2**)		Citronellol (**3**)	
					Sp. act.^{c)} dpm/mmol	Incorp. %	Sp. act.^{c)} dpm/mmol	Incorp. %
12	Leu-U-^{14}C	(0.01)	12	Oct.	5.34×10^{3}	0.0011	8.50×10^{2}	0.0002
13	Leu-U-^{14}C	(0.07)	24	Oct.	1.13×10^{4}	0.0028	5.15×10^{3}	0.0017
14	Leu-U-^{14}C	(0.01)	72	Oct.	1.20×10^{4}	0.0036	1.40×10^{4}	0.0032
15	Val-U-^{14}C	(0.05)	24	Oct.	3.10×10^{4}	0.0009	2.84×10^{3}	0.0009
16	Ala-2-^{14}C	(0.03)	24	Aug.	3.86×10^{5}	0.024	1.85×10^{4}	0.014
17	NaOAc-2-^{14}C	(0.083)	12	July	5.67×10^{5}	0.016	1.48×10^{5}	0.0060
18	NaOAc-2-^{14}C	(0.083)	24	July	1.11×10^{6}	0.027	2.15×10^{5}	0.0091
19	MVA-2-^{14}C	(0.02)	24	Aug.	1.12×10^{4}	0.010^{d)}	4.76×10^{3}	0.0054^{d)}
20	MVA-2-^{14}C	(0.02)	24	Sep.	1.53×10^{4}	0.016^{d)}	1.59×10^{4}	0.018^{d)}

a), b), and c) Refer to a), b), and c) in Table 1 respectively. d) Calculated as the only (3*R*)-enantiomer of a racemic mixture of the substrate participates in the formation of **2** and **3**.

Table 3. Radioactivity in linalool (**1**) and its degradation products after uptake of L-leucine-U-^{14}C, L-valine-U-^{14}C, and DL-mevalonic-2-^{14}C acid

Compound (carbons originating from **1**)	Specific radioactivity, dpm/mmol				
	Exptl 1^{a)} (Leu-U-^{14}C)^{b)}	Exptl 2 (Leu-U-^{14}C)	Exptl 3 (Val-U-^{14}C)	Exptl 5 (Val-2-^{14}C)	Exptl 11 (MVA-2-^{14}C)
Linalool (**1**) (C-1—C-10)	1.40×10^{2}	1.70×10^{2}	7.52×10^{2}	2.72×10^{2}	3.15×10^{3}
Hexanolide (**5**) (C-1—C-6 and C-9)	7.37×10	8.40×10	4.36×10^{2}	1.69×10^{2}	2.05×10^{3}
Acetone (C-7, C-8, and C-10)	7.01×10	8.62×10	3.17×10^{2}	1.06×10^{2}	9.50×10^{2}

a) "Exptl No." correspond to the numbers in Table 1. b) Refer to a) in Table 1.

derivative respectively; these products were purified to a constant specific radioactivity by recrystallization. Table 3 shows the radioactivities of these degradation products. In the uptake of leucine-U-^{14}C and valine-U-^{14}C, the atoms of their tracer element are uniformly located on all the carbon atoms of DMAPP and IPP; therefore, the 5/3 of the total radioactivity of acetone resides on the DMAPP-derived moiety (C-5—C-8 and C-10), and the radioactivity of the IPP-derived moiety (C-1—C-4 and C-9) must be the difference between the radioactivity of **5** and 2/3 that of acetone. On the other hand, it has been established that the C-4 and C-8 carbon atoms of the monoterpenoids are labeled with the tracer from MVA-2-^{14}C.[22,23] In this

TABLE 4. THE DISTRIBUTION OF RADIOACTIVITY IN THE IPP- AND THE DMAPP-DERIVED MOIETIES OF LINALOOL (**1**), GERANIOL (**2**), AND CITRONELLOL (**3**) BIOSYNTHESIZED FROM THE RADIOISOTOPICALLY LABELED PRECURSORS

Exptl No.[a]	Compound	Precursors[b]	Distribution/%	
			IPP-M.[c]	DMAPP-M.[c]
1	Linalool (**1**)	Leu-U-^{14}C	19	81
2		Leu-U-^{14}C	16	84
3		Val-U-^{14}C	30	70
5		Val-U-^{14}C	36	64
6		Ala-2-^{14}C	57	43
7		Ala-2-^{14}C	56	44
8		NaOAc-2-^{14}C	51	49
11		MVA-2-^{14}C	68	32
13	Geraniol (**2**)	Leu-U-^{14}C	23	72
15		Val-U-^{14}C	16	84
16		Ala-2-^{14}C	64	36
18		NaOAc-2-^{14}C	65	35
19		MVA-2-^{14}C	75	25
13	Citronellol (**3**)	Leu-U-^{14}C	17	83
15		Val-U-^{14}C	12	88
16		Ala-2-^{14}C	63	37
18		NaOAc-2-^{14}C	58	42
19		MVA-2-^{14}C	80	20

a) "Exptl No." correspond to the numbers in Tables 1 and 2. b) Refer to a) in Table 1. c) IPP-M. and DMAPP-M. denote the IPP- and DMAPP-derived moieties respectively.

case, therefore, all the radioactivity located on the DMAPP- and IPP-derived moieties would appear in acetone and **5**.

On the basis of the radioactivity of acetone and **5**, the distributions of radioactivity in the IPP- and DMAPP-derived moieties of **1** were determined by proportional allotment; they are shown in Table 4. The DMAPP-derived moiety of **1** was labeled with 81—84% of the total radioactivity incorporated into **1** in the administration of leucine-U-^{14}C and with 64—70% of it in the case of valine-U-^{14}C, whereas the DMAPP-derived moiety of **1** biosynthesized from MVA-2-^{14}C was labeled with less than 32% of the total activity.

In order to confirm the contrasted localization of the radioactivity on the IPP- and DMAPP-derived moieties between the incorporations of leucine and mevalonate, a mixture of leucine-4,5-^{3}H and MVA-2-^{14}C was fed to the plant to give a ^{3}H- and ^{14}C-labeled sample of **1**. The linalool (**1**) was then degraded to levulinic acid and acetone in the same manner as above. The tracer originating from leucine-4,5-^{3}H would be located on the C-4 and C-9 and the C-8 and C-10 carbon atoms; the former pair of carbon atoms constitutes a part of the IPP-derived moiety, and the latter, a part of the DMAPP-derived moiety. On the other hand, the tracer originating from MVA-2-^{14}C is located at the C-4 and the C-8 carbon atoms, which reside on the IPP- and DMAPP-derived moieties respectively. In the incorporation of the mixture of ^{3}H-labeled leucine and ^{14}C-labeled MVA, therefore, all the radioactivities located on the DMAPP- and IPP-derived moieties would appear in molecules of acetone and levulinic acid respectively. The distributions of the radioactivity of these tracers and the ^{3}H/^{14}C ratio of the radioactivity in molecules of the degradation products are shown in Table 5. Although the ^{3}H/^{14}C ratio in the whole molecule of **1** was 0.77, the ratios in the IPP- and DMAPP-derived moieties were 0.45 and 1.18 respectively. This fact indicates that the distribution of ^{3}H originating from leucine was greater in the DMAPP-derived moiety than in the IPP-derived moiety, whereas the distribution of ^{14}C originating from MVA was greater in the IPP-derived moiety than in the DMAPP-derived moiety. Thus, the experiment of the simultaneous feeding of ^{3}H-labeled leucine and ^{14}C-labeled MVA has firmly established the occurrence of the contrasted unbalanced distribution of radioactivity in the IPP- and DMAPP-derived moieties of the monoterpenoids when leucine and MVA are separately incorporated into the monoterpenoids.[3—6,14—17]

TABLE 5. RADIOACTIVITY IN LINALOOL (**1**) AND ITS DEGRADATION PRODUCTS AFTER UPTAKE OF A MIXTURE OF DL-LEUCINE-4,5-^{3}H AND DL-MVA-2-^{14}C TO *C. camphora*

Compound (carbons originating from **1**)	Specific radioactivity		^{3}H/^{14}C ratio
	^{3}H dpm/mmol	^{14}C dpm/mmol	
Linalool (**1**) (C-1—C-10)	2.18×10^{3}	2.85×10^{3}	0.77
Levulinic acid (C-3—C-6 and C-9)	0.74×10^{3}	1.66×10^{3}	0.45
Acetone (C-7, C-8, and C-10)	1.43×10^{3}	1.21×10^{3}	1.18

*The Distribution of Radioactivity in Geraniol (**2**) and Citronellol (**3**) Biosynthesized from ^{14}C-Labeled Leucine, Valine, and MVA.* Radioactive samples of geraniol (**2**) and citronellol (**3**), resulting from the uptake of leucine-U-^{14}C, valine-U-^{14}C, and MVA-2-^{14}C, were subjected to permanganate-periodate oxidation. Geraniol (**2**) was degraded to levulinic acid containing the C-3—C-6 and C-9 carbon atoms and to acetone containing the C-7, C-8, and C-10 carbon atoms. Citronellol (**3**) was degraded to 6-hydroxy-4-methylhexanoic acid (**6**) containing the C-1—C-6 and C-9 carbon atoms and to acetone containing the C-7, C-8, and C-10 carbon atoms. The radioactivities of these compounds are listed in Tables 6 and 7. The distribution of the radioactivity of the ^{14}C-tracer in the IPP- and DMAPP-derived moieties of **2** and **3** were determined by the proportional allotment in the same manner as in the case of **1**; they are shown in Table 4. The uptake of the radioisotopically labeled amino acids resulted in the location of 72—88% of the total radioactivities on the DMAPP-derived moiety, whereas the predominant radioactivity resided on the IPP-derived moiety in the uptake of the ^{14}C-labeled MVA.

*The Distribution of Radioactivity in Linalool (**1**), Geraniol (**2**), and Citronellol (**3**) Biosynthesized from ^{14}C-Labeled Alanine, Acetate, and MVA.* The distribution of radioactivity in the IPP- and DMAPP-derived moieties of **1**, **2**, and **3**, biosynthesized from alanine-2-^{14}C,

TABLE 6. RADIOACTIVITY IN GERANIOL (**2**) AND ITS DEGRADATION PRODUCTS AFTER UPTAKE OF THE RADIOISOTOPICALLY LABELED PRECURSORS

Compound (carbons originating from **2**)	Specific radioactivity, dpm/mmol				
	Exptl 13[a] (Leu-U-^{14}C)[b]	Exptl 15 (Val-U-^{14}C)	Exptl 16 (Ala-2-^{14}C)	Exptl 18 (NaOAc-2-^{14}C)	Exptl 19 (MVA-2-^{14}C)
Geraniol (**2**) (C-1—C-10)	1.84×10^{3}	3.76×10^{2}	2.95×10^{3}	9.71×10^{4}	1.24×10^{3}
Levulinic acid (C-3—C-6 and C-9)	8.32×10^{2}	1.86×10^{2}	1.48×10^{3}	5.31×10^{4}	9.33×10^{2}
Acetone (C-7, C-8, and C-10)	7.89×10^{2}	1.90×10^{2}	5.27×10^{2}	2.25×10^{4}	3.08×10^{2}

a) "Exptl No." correspond to the numbers in Table 2. b) Refer to a) in Table 1.

TABLE 7. RADIOACTIVITY IN CITRONELLOL (**3**) AND ITS DEGRADATION PRODUCTS AFTER UPTAKE OF THE RADIOISOTOPICALLY LABELED PRECURSORS

Compound (carbons originating from **3**)	Specific radioactivity, dpm/mmol				
	Exptl 13[a] (Leu-U-^{14}C)[b]	Exptl 15 (Val-U-^{14}C)	Exptl 16 (Ala-2-^{14}C)	Exptl 18 (NaOAc-2-^{14}C)	Exptl 19 (MVA-2-^{14}C)
Citronellol (**3**) (C-1—C-10)	1.25×10^{3}	1.77×10^{3}	1.03×10^{3}	1.45×10^{4}	1.52×10^{2}
6-Hydroxy-4-methylhexanoic acid (**6**) (C-1—C-6 and C-9)	6.27×10^{2}	9.38×10^{2}	8.40×10^{2}	1.01×10^{4}	1.12×10^{2}
Acetone (C-7, C-8, and C-10)	6.20×10^{2}	8.36×10^{2}	1.90×10^{2}	3.97×10^{3}	3.08×10

a) "Exptl No." correspond to the numbers in Table 2. b) Refer to a) in Table 1.

TABLE 8. RADIOACTIVITY IN LINALOOL (**1**) AND ITS DEGRADATION PRODUCTS AFTER UPTAKE OF DL-ALANINE-2-^{14}C, SODIUM ACETATE-2-^{14}C, AND DL-MEVALONIC-2-^{14}C ACID

Compound (carbons originating from **1**)	Specific radioactivity, dpm/mmol			
	Exptl 6[a] (Ala-2-^{14}C)[b]	Exptl 7 (Ala-2-^{14}C)	Exptl 8 (NaOAc-2-^{14}C)	Exptl 10 (MVA-2-^{14}C)
Linalool (**1**) (C-1—C-10)	4.25×10^{3}	4.46×10^{3}	1.10×10^{4}	4.39×10^{4}
4-Methyl-4-hexanolide (**5**) (C-1—C-6 and C-9)	—	3.25×10^{3}	—	2.68×10^{4}
Formaldehyde (C-1)	9.37×10^{2}	—	3.78×10^{2}	1.37×10^{3}
Levulinic acid (C-3—C-6 and C-9)	2.53×10^{3}	—	4.90×10^{3}	2.85×10^{4}
Iodoform (C-9)	2.67×10	—	—	9.70×10^{2}
Succinic acid (C-3—C-6)	2.35×10^{3}	—	—	2.77×10^{4}
Acetone (C-7, C-8, and C-10)	1.10×10^{3}	9.90×10^{2}	3.21×10^{3}	1.70×10^{4}
Iodoform (C-8 and/or C-10)	—	—	—	8.07×10^{3}

a) "Exptl No." correspond to the numbers in Table 1. b) Refer to a) in Table 1.

acetate-2-^{14}C, and MVA-2-^{14}C, was determined by subjecting them to degradation as follows. The oxidation of **1** with permanganate-periodate gave levulinic acid, acetone, and formaldehyde, which contain the C-3—C-6 and C-9 carbon atoms, the C-7, C-8, and C-10 carbon atoms, and the C-1 carbon atom respectively. A part of the sample of levulinic acid was further degraded by an iodoform reaction to iodoform containing the C-9 carbon atom and to succinic acid containing the C-3—C-6 carbon atoms. Also, **1** was, on partial hydrogenation, converted to 1,2-dihydrolinalool (**4**), which was then degraded to 4-methyl-4-hexanolide (**5**) and acetone by permanganate-periodate oxidation. Table 8 shows the radioactivities of **1** and these degradation products. By oxidation with this reagent, geraniol (**2**) was degraded to levulinic acid and acetone, and citronellol (**3**), to 6-hydroxy-4-methylhexanoic acid (**6**) and acetone. The radioactivities of **2** and **3** and the degradation products are shown in Tables 6 and 7.

Table 8 indicates that 22% of the radioactivity due to the tracer originating from alanine-2-^{14}C is located on the C-1 carbon atom of **1** and 26% on the portion composed of the C-7, C-8, and C-10 carbon atoms, while the C-9 carbon atom is unlabeled. These results indicate that the tracer from alanine-2-^{14}C resides on the C-1, C-3, C-5, and C-7 carbon atoms. Tables 6 and 7 indicate that the labeling pattern of

2 and **3** is the same as that of **1**. In the uptake of alanine-2-^{14}C, therefore, the radioactivity of the DMAPP-derived moiety (C-5—C-8 and C-10) of **1**, **2**, and **3** is double that of acetone. On the other hand, the radioactivity of the IPP-derived moiety (C-1—C-4 and C-9) of **1** and **2** was evaluated by doubling the difference between the radioactivities of levulinic acid and acetone; in addition, the radioactivity of the IPP moiety of **1** was evaluated by deducting the radioactivity of acetone from that of 4-methyl-4-hexanolide (**5**). With respect to **3**, the radioactivity of its IPP-derived moiety was evaluated by deducting the radioactivity of acetone from that of 6-hydroxy-4-methylhexanoic acid (**6**). In the uptake of acetate-2-^{14}C, it has been well established that the tracer resides on the C-2, C-4, C-6, and C-8—C-10 carbon atoms of the acyclic monoterpenoids.[24–27] Taking this labeling pattern into consideration, the distribution of radioactivity in the IPP- and DMAPP-derived moieties of **1**, **2**, and **3** was evaluated by the proportional allotment of the radioactivities of acetone and levulinic acid in a manner similar to that described above.

On the basis of the specific radioactivities of the IPP- and DMAPP-derived moieties determined as above, the distribution of radioactivity in these moieties was evaluated; it is shown in Table 4. The incorporation of ^{14}C-labeled alanine and acetate into geraniol (**2**) and citronellol (**3**) resulted in the preferential location of radioactivity on the IPP-derived moiety, similarly to the incorporation of ^{14}C-labeled MVA, but in opposition to the incorporation of ^{14}C-labeled leucine and valine. The localization of radioactivity in linalool (**1**) biosynthesized from ^{14}C-labeled alanine was predominant on its IPP-derived moiety, but when **1** was biosynthesized from ^{14}C-labeled acetate, the imbalance of the distribution of radioactivity on the IPP- and DMAPP-derived moieties was very small.

Discussion

The previous reports[3,9] described that leucine and valine are not precursors for the monoterpenoid biosynthesis in higher plants because of their negligible incorporation into monoterpenoids. However, it has been found that the radioisotopically labeled leucine and valine are surely incorporated into monoterpenoids, though the incorporation is at a low level, as is shown in Tables 1 and 2. The low incorporation of the amino acids may be explained in terms of the operation of several factors: (i) the radioactivity of the amino acids is scattered into other metabolites rather than the monoterpenoids, (ii) there is a great pool of the amino acids, so that their radioactivity is diluted, and (iii) the low permeation of the amino acids into the biosynthetic site of monoterpenoids causes the low incorporation.

The DMAPP-derived moiety of the monoterpenoids, **1**, **2**, and **3**, biosynthesized from leucine-U-^{14}C and valine-U-^{14}C was labeled with more than 64% of the incorporated tracers, whereas this moiety of the monoterpenoids biosynthesized from MVA-2-^{14}C contained less than 32% of the tracers, as is shown in Table 4. If the monoterpenoids are biosynthesized from the amino acids *via* MVA, the distribution pattern will be similar to the pattern in the monoterpenoids biosynthesized from MVA-2-^{14}C. This indicates that, although leucine and valine have a carbon-skeleton similar to that of MVA, these amino acids are incorporated into the monoterpenoids, not *via* MVA, but by an alternate route. On the other hand, the distribution pattern in the monoterpenoids biosynthesized from alanine-2-^{14}C was similar to that in the monoterpenoids biosynthesized from MVA-2-^{14}C and acetate-2-^{14}C. This indicates that alanine is incorporated into the monoterpenoids by a route *via* MVA. Thus, it is likely that administered leucine and valine participate in the biosynthesis of the monoterpenoids by their direct conversion to DMAPP through an alternate route rather than through the mevalonate pathway, whereas administered alanine is first metabolized to acetyl-CoA, which then constructs preferentially the IPP-derived moiety of the monoterpenoids *via* MVA.

Experimental

The radioisotopically labeled monoterpenoids and their degradation products were purified to a constant specific radioactivity by a combination of preparative TLC and/or repeated recrystallization of the crystalline derivatives in every case that these could be prepared. The melting points and spectral data of all the products agreed with those of authentic samples.[22,23] The TLC analyses were carried out using silica-gel plates (Kieselgel 60 G; 0.25 mm thick) and the following five kinds of solvents: (i) hexane/EtOAc=9/1, (ii) hexane/EtOAc=4/1, (iii) hexane/EtOAc=1/1, (iv) $CHCl_3$, and (V) MeOH/acetone/formic acid=70/10/1. Spots on the plates were visualized by vaniline-H_2SO_4 spraying and subsequent heating to 120 °C. The preparative TLC was performed on silica-gel plates (Kieselgel 60 G; 0.75 mm thick) or on 3% $AgNO_3$–silica-gel plates (Kieselgel 60 G; 0.75 mm thick) with the above-described solvents. The GLC analyses were performed on an instrument equipped with FID and a glass column (2.0 m × 3 mm) packed with either 2% OV-17, 15% DEGS, or 10% PEG-20M on Chromosorb W (80—100 mesh). The radioactivity was measured on a liquid-scintillation spectrometer using Bray's scintillation solvent.[19] The counting error was within 2%.

Plant Materials and Labeled Precursors. The leaves of *Cinnamomum camphora* Sieb. var. *linalooliferum* Fujita, which had been grown outdoors on the campus of Hiroshima University, produced an essential oil containing up to 90% (wt) of linalool (**1**) in August. The linalool (**1**) isolated showed $[\alpha]_D^{25}$ $-19.3°$ (c 13.3, $CHCl_3$) and n_D^{25} 1.4603. Each top branch used for the feeding experiments was *ca.* 10 cm in length and *ca.* 5 g in fresh wt. The leaves of *Pelargonium roseum* Bourbon, which had been wintered indoors and grown outdoors from spring to autumn, produced an essential oil containing 35—40% of geraniol (**2**) and 25—30% of citronellol (**3**) in August. The isolated geraniol (**2**) and citronellol (**3**) showed n_D^{25} values of 1.4760 and 1.4551 respectively. Each top branch of the plant used for feeding experiments was *ca.* 15 cm long and *ca.* 10 g in fresh wt.

DL-Mevalonic-2-^{14}C acid (MVA-2-^{14}C) (7.16 mCi/mmol), L-leucine-U-^{14}C (Leu-U-^{14}C) (251 mCi/mmol), L-valine-U-^{14}C (Val-U-^{14}C) (225 mCi/mmol), DL-alanine-2-^{14}C (Ala-2-^{14}C) (12 mCi/mmol), and sodium acetate-2-^{14}C (58 mCi/

mmol) used for the feeding experiments were products of the Daiichi Pure Chemicals Co., Ltd., Tokyo.

Administration of the Labeled Precursors and Isolation of the Radioisotopically Labeled Monoterpenoids. The labeled precursor, dissolved in a phosphate-buffered solution (0.5 cm^3; pH 7.4), was fed to the fresh cuttings (50 g in total wt) through their cut-stem in a glass tube, and then distilled water was soaked into the cuttings several times to complete the uptake of the labeled precursor. At the end of the metabolic periods shown in Tables 1 and 2, the cuttings were steam-distilled. The distillate was extracted with four 50-cm^3 portions of ether. The combined ether layer was washed with two 50-cm^3 portions of a 5% sodium hydrogencarbonate solution and water, and then dried over anhydrous sodium sulfate. The removal of the solvent from the ether layer at temperature below 40 °C afforded a crude essential oil. The essential oil (0.6—1.0 g) obtained from *C. camphora* was subjected to preparative TLC with solvent (i) to give linalool (**1**), which was then further purified by TLC. The essential oil (0.15—0.2 g) obtained from *P. roseum* was separated on $AgNO_3$–silica-gel plates with solvent (ii) to give geraniol (**2**) and citronellol (**3**), which were subsequently further purified by TLC with silica-gel plates and solvent (i). The homogeneity of these monoterpene alcohols was confirmed by GLC analyses using a 2% OV-17 column at 100 °C, a 15% DEGS column at 120 °C, and a 10% PEG 20 M column at 120—180 °C, in addition to the examination of the constant specific radioactivity.

For time-course studies of the incorporation of the tracers into the monoterpene alcohols, each precursor was fed to the cuttings of the plant in the same manner as above, and then the cuttings were steam-distilled at appropriate time intervals to give the essential oil. This oil was subjected to isolation of the monoterpene alcohol in the same way as above.

*Degradation of Linalool (**1**).* Following the reported procedure of the $KMnO_4$–$NaIO_4$ oxidation,[20] a suspension of linalool (**1**) (350 mg) in a solution of $NaIO_4 \cdot H_2O$ (2.6 g) in water (260 cm^3) was stirred for 10 min at room temp. The mixture was neutralized with sodium carbonate under cooling to 0—10 °C. To the neutralized solution, $KMnO_4$ (250 mg) dissolved in water (15 cm^3) was added drop by drop, and, after which, the mixture was stirred overnight below 10 °C. The mixture, after the reduction of the remaining oxidant with sodium hydrogensulfite, was steam-distilled. The distillate (5 cm^3) was divided into two portions. One portion (2.5 cm^3) was treated with thiosemicarbazide as usual to give acetone thiosemicarbazone (64 mg), which was then purified by a combination of preparative TLC with silica gel and solvent (iii) and repeated recrystallizations; it showed mp and mixed mp 179.0—179.5 °C. The other portion (2.5 cm^3) was added to a solution of dimedone in water to give the bisdimedone derivative of formaldehyde (23 mg; mp and mixed mp 187—188 °C).[28]

The steam-distillation residue was concentrated to 100 cm^3, acidified with diluted H_2SO_4, and extracted, after the reduction of the remaining oxidant with sodium hydrogensulfite, with ether using a liquid-liquid continuous extractor to give an acidic product. This acidic product was subjected to preparative TLC with silica gel and solvent (v) to give levulinic acid (79 mg). This acid, on methylation with CH_2N_2, was converted to methyl levulinate (75 mg), which was subsequently further purified by TLC with silica gel and solvent (ii). The purified levulinate was divided into two portions. One portion (25 mg) was treated with an aqueous solution of thiosemicarbazide to give methyl levulinate thiosemicarbazone (38 mg; mp and mixed mp 140—141 °C). The other portion (25 mg) was subjected to hypoiodite oxidation to give iodoform (17 mg; mp and mixed mp 119—120 °C) and succinic acid (7 mg; mp and mixed mp 186—188 °C). The radioactivities of these products are shown in Tables 5 and 8.

*Degradation of Geraniol (**2**).* Radioactive geraniol (**2**) (20 mg) isolated was diluted with the carrier (280 mg) and then degraded to acetone and levulinic acid with $KMnO_4$–$NaIO_4$ in the same manner as above. The acetone was converted to the thiosemicarbazone derivative (72 mg; mp and mixed mp 179—180 °C). The levulinic acid (110 mg) was converted to methyl levulinate (86 mg), which was then transformed to the thiosemicarbazone derivative (116 mg; mp and mixed mp 140—141 °C). Table 6 shows the radioactivities of geraniol and these products.

*Degradation of Citronellol (**3**).* Radioactive citronellol (**3**) (20 mg) isolated was diluted with the carrier (300 mg) and degraded to acetone and 4-methyl-6-hydroxyhexanoic acid (**6**) by $KMnO_4$–$NaIO_4$ oxidation in the same manner as above. The acetone was converted to the thiosemicarbazone derivative (68 mg; mp and mixed mp 179—180 °C). The 4-methyl-6-hydroxyhexanoic acid (**6**)[22] [110 mg; IR (liq.) 3500—2900 and 1710 cm^{-1} (COOH)] was, on treatment with CH_2N_2, converted to its methyl ester [IR (liq.) 3450 (OH) and 1741 cm^{-1} (ester C=O); ^{1}H-NMR $\delta=0.93$ (3H, d, $J=5.8$ Hz, CH_3), 2.33 (2H, bt, $J=6.0$ Hz, $-C\underline{H}_2-COOCH_3$), 3.65 (3H, s, $COOCH_3$), and 3.67 (2H, t, $J=6.1$ Hz, $-C\underline{H}_2OH$); MS (70 eV), m/z (rel intensity), 160 (M^+, 22), 142 (12), 129 (23), 87 (88), 74 (83), 69 (84), 55 (100), 43 (73), and 41 (95)]. The specific radioactivities of citronellol and the degradation products are shown in Table 7.

*Degradation of Dihydrolinalool (**4**).* Purified, radioactive linalool (**1**) (350 mg), dissolved in absolute MeOH (3 cm^3), was selectively hydrogenated in the presence of Adams PtO_2 (4 mg). The purification of the reaction mixture by preparative TLC, using $AgNO_3$–silica-gel plates and solvent (i), gave 1,2-dihydrolinalool (**4**) (300 mg), which was then subjected to $KMnO_4$–$NaIO_4$ oxidation as described above. The reaction mixture was steam-distilled. The distillate (5 cm^3) was divided into two portions. One portion (2.5 cm^3) was treated with an aqueous solution of thiosemicarbazide as usual to give acetone thiosemicarbazone (105 mg; mp and mixed mp 179—180 °C). Another portion (2.5 cm^3) was subjected to hypoiodite oxidation to give iodoform (80 mg; mp and mixed mp 119—120 °C). On the other hand, a steam-distillation residue was concentrated to 100 cm^3, acidified with 5% HCl, and extracted with ether to give 4-methyl-4-hexanolide (**5**)[23] [80 mg; IR (liq.) 1765 (C=O of γ-lactone) and 1238 and 1161 cm^{-1} (C–O of the lactone); ^{1}H-NMR ($CDCl_3$) $\delta=0.97$ (3H, t, $J=7.0$ Hz, CH_3), 1.38 (3H, s, CH_3), and 1.73 (2H, q, $J=7.0$ Hz, $-CH_2-$)]. The hexanolide (**5**), dissolved in EtOH (1 cm^3), was refluxed with 1 M† NaOH (1.5 cm^3) for 1 h. To this reaction mixture, after neutralization, we added *S*-benzylthiouronium urea (160 mg) dissolved in EtOH (1 cm^3) to give the *S*-benzylthiouronium salt (150 mg; mp 132—133 °C). The radioactivities of linalool and its degradation products are shown in Tables 3 and 8.

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† 1 M=1 mol dm^{-3}.

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