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BIOSYNTHESIS OF MONOTERPENOIDS IN HIGHER PLANTS. LOCALIZATION OF RADIOACTIVITY IN FAVOR OF THE ISOPENTENYL PYROPHOSPHATE-DERIVED MOIETY OF MONOTERPENOIDS IN THE UPTAKE OF ALANINE-2-^{14}C

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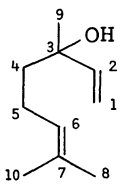
Uptake of DL-alanine-2-^{14}C to the twigs of *Cinnamomum Camphora* Sieb. and *Pelargonium roseum* Bourbon resulted in the preferential location of radioactivity on the isopentenyl pyrophosphate-derived moiety of linalool, geraniol, and citronellol, in opposition to incorporations of leucine and valine into these monoterpenoids. The biosynthetic pathway of the monoterpenoids from the amino acid is discussed.

In the biosynthesis of monoterpenoids from mevalonic(MVA)-2-^{14}C acid in higher plants, many examples of the predominant location of radioactivity in the isopentenyl pyrophosphate(IPP)-derived moiety of the monoterpenoids are described,[1-4] whereas we recently found the unbalanced location of radioactivity in favor of the dimethylallyl pyrophosphate(DMAPP)-derived moiety when ^{14}C-labeled leucine and valine were incorporated into monoterpenoids, and proposed the possibility of a non-mevalonoid route for the monoterpenoid biosynthesis from such amino acids.[5-7] We now have examined the incorporation of ^{14}C-labeled alanine into linalool (I) in *Cinnamomum Camphora* Sieb. var. *linalooliferum* Fujita, and geraniol (II) and citronellol (III) in *Pelargonium roseum* Bourbon, and found a new fact differing from the cases of leucine and valine. Here we wish to communicate this finding.

Feeding experiments of DL-alanine-2-^{14}C were carried out in the same way as done in uptakes of leucine and valine. Simultaneously the uptake of DL-MVA-2-^{14}C was



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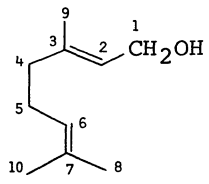

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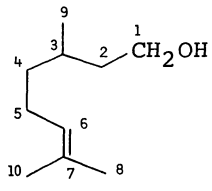

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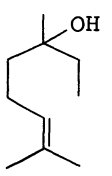

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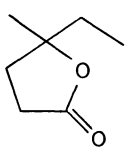

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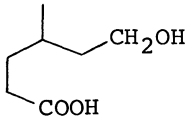

VI

TABLE 1. INCORPORATION OF THE RADIOACTIVE TRACER INTO LINALOOL (I), GERANIOL (II), AND CITRONELLOL (III)

Exp. No.	Compds	Precursors[a] (mCi)	Seasons	time[b] (hr)	Sp. radioactivity[c] (dpm/mmole)	Incorporation (%)[d]
1	I	Ala-^{14}C (0.05)	June	24	1.86×10^4	0.076
2	"	" (0.02)	July	24	3.00×10^3	0.026
3	"	MVA-^{14}C (0.10)	June	24	4.39×10^4	0.22
4	II	Ala-^{14}C (0.03)	Aug.	24	3.86×10^5	0.024
5	"	MVA-^{14}C (0.02)	Sept.	24	1.53×10^4	0.016
6	III	Ala-^{14}C (0.03)	Aug.	24	1.85×10^4	0.014
7	"	MVA-^{14}C (0.02)	Sept.	24	1.59×10^4	0.018

a) Ala-^{14}C and MVA-^{14}C denote DL-alanine-2-^{14}C and DL-mevalonic-2-^{14}C acid, respectively.
b) Metabolic period after uptake of the tracer.
c) Specific radioactivity in dpm/mmole. Values cannot be compared in different experiments as different quantities of carrier and/or tracer were used.
d) Incorporations were calculated with respect to only L-alanine and L-MVA.

carried out for comparison with that of alanine. A phosphate-buffered solution (pH 7.4) containing the ^{14}C-labeled precursor was fed through a cut-stem into the leaves and stems, which then were steam-distilled. An ethereal extract of the distillate on preparative TLC (silica gel) gave linalool (I) from *C. Camphora* and geraniol (II) and citronellol (III) from *P. roseum*. Radioactivities of I, II, and III were measured in a liquid scintillation spectrometer using Bray's scintillation solvent[8] and are shown in Table 1. This table shows that the incorporation of alanine into these monoterpenoids is higher than that of leucine and valine.[5-7]

To determine labeling patterns in I, II, and III, these were subjected to the following degradation. Oxidation of I with permanganate-periodate[9] gave levulinic acid, acetone, and formaldehyde, which originate from the C-3∿C-6 and C-9, the C-7, C-8, and C-10, and the C-1 moieties, respectively. A part of a sample of levulinic acid was further subjected to the iodoform reaction to give iodoform containing the carbon atom of C-9 and succinic acid containing the carbon atoms of C-3∿C-6. Also, I was converted into 1,2-dihydrolinalool (IV) by selective hydrogenation (PtO_2), followed by degradation by permanganate-periodate oxidation into 4-methyl-4-hexanolide (V) containing C-1∿C-6 and C-9 and acetone containing C-7, C-8, and C-10. The γ-lactone (V) was purified by its conversion to the crystalline S-benzylthiouronium salt derivative.[10] II was degraded into levulinic acid and acetone by permanganate-periodate oxidation. III on permanganate-periodate oxidation gave 6-hyroxy-4-methylhexanoic acid (VI) containing C-1∿C-6 and C-9 and acetone containing C-7, C-8, and C-10. Purification of this acid (VI) was effected by its conversion to the methyl ester. The degradation products and their derivatives were purified to constant specific radioactivity by a combination of recrystallization, chromatography, and/or sublimation to determine their radioactivity, as shown in Tables 2-4.

Detailed studies on the labeling pattern of I biosynthesized from alanine-2-^{14}C revealed the location of 22 % of the radioactivity of the incorporated tracer on C-1 and 26 % on a portion composed of C-7, C-8, and C-10 and the unlabeling of the C-9

TABLE 2. DISTRIBUTION OF RADIOACTIVITY IN LINALOOL (I) AFTER UPTAKE OF THE TRACER

Compds (Carbons originated from I)	Sp. radioactivity (dpm/mmole)[a]		
	Exp. 1	Exp. 2	Exp. 3
Linalool (C-1∿C-10)	4.25×10^3	4.46×10^3	4.39×10^4
4-Methyl-4-hexanolide (C-1∿C-6 and C-9)	———	3.47×10^3	2.68×10^4
Formaldehyde (C-1)	9.37×10^2	———	1.37×10^3
Levulinic acid (C-3∿C-6 and C-9)	2.53×10^3	———	2.85×10^4
Iodoform (C-9)	2.67×10^2	———	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	1.70×10^4
Iodoform (C-8 and/or C-10)	———	———	8.07×10^3

a) "Exp. No." corresponds to the number in Table 1.

TABLE 3. DISTRIBUTION OF RADIOACTIVITY IN GERANIOL (II) AFTER UPTAKE OF THE TRACER

Compds (Carbons originated from II)	Sp. radioactivity (dpm/mmole)[a]	
	Exp. 4	Exp. 5
Geraniol (C-1∿C-10)	2.95×10^3	1.24×10^3
Levulinic acid (C-3∿C-6 and C-9)	1.48×10^3	9.33×10^2
Acetone (C-7, C-8, and C-10)	5.27×10^2	3.08×10^2

a) "Exp. No." corresponds to the number in Table 1.

TABLE 4. DISTRIBUTION OF RADIOACTIVITY IN CITRONELLOL (III) AFTER UPTAKE OF THE TRACER

Compds (Carbons originated from III)	Sp. radioactivity (dpm/mmole)[a]	
	Exp. 6	Exp. 7
Citronellol (C-1∿C-10)	1.03×10^3	1.52×10^2
6-Hydroxy-4-methylhexanoic acid (C-1∿C-6 and C-9)	8.40×10^2	1.12×10^2
Acetone (C-7, C-8, and C-10)	1.90×10^2	3.08×10

a) "Exp. No." corresponds to the number in Table 1.

carbon atom (Table 2). This result indicates that the tracer from alanine-2-^{14}C resides at C-1, C-3, C-5, and C-7 of I. Labeling at the same positions is also expected for II and III (cf. Tables 3 and 4). In the uptake of alanine-2-^{14}C, thus, the DMAPP-derived moiety (C-5∿C-8 and C-10) should be contain double the radioactivity of acetone, and the radioactivity in the IPP-derived moiety (C-1∿C-4 and C-9) are able to be calculated by using double the difference in the radioactivity between levulinic acid and acetone and the difference in the activity between 4-methyl-4-hexanolide and acetone and between 6-hydroxy-4-methylhexanoic acid and acetone. On the other hand, the tracer from MVA-2-^{14}C is located on C-4 and C-8 of the monoterpenoids. In the uptake of MVA-2-^{14}C, thus, all the radioactivity located on the DMAPP-derived moiety appear in acetone and all the activity on the IPP-derived moiety in levulinic acid,

TABLE 5. DISTRIBUTION OF RADIOACTIVITY IN IPP- AND DMAPP-DERIVED MOIETIES OF LINALOOL (I), GERANIOL (II), AND CITRONELLOL (III)

Exp. No.[a]	Compds	Precursors[b]	Sp. radioact. (dpm/mmole)		Distribution (%)	
			IPP	DMAPP	IPP	DMAPP
1	I	Ala-2-^{14}C	2.86×10^3	2.20×10^3	57	43
2	"	"	2.48×10^3	1.98×10^3	56	44
3	"	MVA-2-^{14}C	2.68×10^4	1.70×10^4	61	39
4	II	Ala-2-^{14}C	1.90×10^3	1.05×10^3	64	36
5	"	MVA-2-^{14}C	9.33×10^2	3.08×10^2	75	25
6	III	Ala-2-^{14}C	6.50×10^2	3.80×10^2	63	37
7	"	MVA-2-^{14}C	1.12×10^2	3.08×10	78	22

a) "Exp. No." corresponds to the number in Table 1.
b) Abbreviations are the same as in Table 1.

4-methyl-4-hexanolide, and 6-hydroxy-4-methylhexanoic acid. On the basis of the labeling patterns (Tables 2-4), specific radioactivities in the IPP- and the DMAPP-derived moieties of I, II, and III were calculated as described above and the distributions were determined by the proportional allotment, as shown in Table 5. The incorporation of labeled alanine into the monoterpenoids resulted in the preferential location of radioactivity on the IPP-derived moiety, similarly to the incorporation of MVA-2-^{14}C but in opposition to the incorporations of labeled leucine and valine.

Thus, it is likely that alanine is first metabolized to acetyl-CoA and converted preferentially to the IPP-derived moiety of monoterpenoids *via* MVA, in contrast to our previous proposal[5-7] that leucine and valine may participate in the biosynthesis of monoterpenoids by their conversion directly to DMAPP through an alternative route rather than the mevalonoid pathway.

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