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BIOSYNTHESIS OF ISOPRENOID FROM AMINO ACID IN HIGHER PLANT. INCORPORATION OF L-LEUCINE AND L-VALINE INTO GERANIOL AND CITRONELLOL

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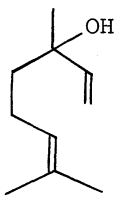
L-[U-^{14}C]Leucine and L-[U-^{14}C]valine were incorporated into geraniol (II) and citronellol (III) in *Pelargonium roseum* Bourbon. The labeling pattern demonstrated that in the higher plant some of the 3,3-dimethylallyl pyrophosphates originate from leucine and valine, not *via* the mevalonoid pathway, but by some alternate route.

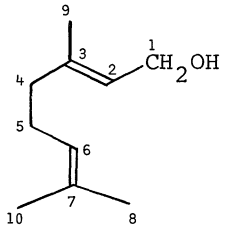
Recently we proposed the possibility of a non-mevalonoid route for the biosynthesis of linalool (I) from amino acids, such as leucine and valine, in higher plant.[1,2] To clarify the route participating in the biosynthesis of monoterpenes, we now have tested incorporations of L-[U-^{14}C]leucine and L-[U-^{14}C]valine into other monoterpenes, such as geraniol (II) and citronellol (III), in *Pelargonium roseum* Bourbon. Here we report evidence for the Biosynthesis of the monoterpenes, (II) and (III), not only by the mevalonoid route but also by the non-mevalonoid route. The incorporation of DL-[2-^{14}C]mevalonic acid (MVA) has also been made for comparison with the uptake of the amino acids.

The ^{14}C-labeled tracers were given to the terminal branches (ca. 15 cm long) of the plant. A phosphate-buffered solution (pH 7.4) of each of L-[U-^{14}C]leucine, L-[U-^{14}C]valine, and DL-[2-^{14}C]MVA was fed through a cut-stem into the leaves and the stems of the plant. The leaves and stems were then subjected to steam-distillation followed by column chromatography and preparative thin-layer chromatography on silica gel to isolate geraniol (II) and citronellol (III). The radioactivity of the terpene alcohols was measured in a Packard Tri-Carb liquid scintillation spectrometer using a Bray's scintillation solvent[3] (Tables 1 and 2). The results show that the terpene alcohols, II and III, were synthesized biologically from L-leucine and L-valine though in a low yield.

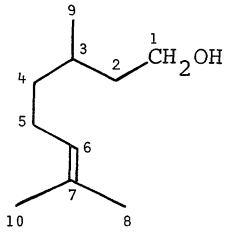
In order to establish the labeling pattern of II and III after the uptake of



I



II



III

TABLE 1. INCORPORATION OF RADIOACTIVE TRACERS INTO GERANIOL (II)

Exp. No.	Precursors* (mCi)	Feeding time (hr)	Seasons	Specific radio-activity of II (dpm/mmole)	Incorporation (%)
1	^{14}C-Leu; 0.04	24	August	1.73×10^4	0.0039
2	^{14}C-Leu; 0.01	12	October	5.34×10^3	0.0011
3	^{14}C-Leu; 0.07	24	"	1.13×10^4	0.0028
4	^{14}C-Leu; 0.01	48	"	1.03×10^4	0.0030
5	^{14}C-Leu; 0.01	72	"	1.23×10^4	0.0036
6	^{14}C-Val; 0.05	24	"	3.10×10^3	0.0009
7	^{14}C-MVA; 0.02	24	August	1.12×10^4	0.0050
8	^{14}C-MVA; 0.02	24	September	1.53×10^4	0.0078

* ^{14}C-Leu, ^{14}C-Val, and ^{14}C-MVA denote L-[U-^{14}C]leucine, L-[U-^{14}C]valine, and DL-[2-^{14}C]mevalonic acid, respectively.

TABLE 2. INCORPORATION OF RADIOACTIVE TRACERS INTO CITRONELLOL (III)

Exp. No.	Precursors* (mCi)	Feeding time (hr)	Seasons	Specific radio-activity of II (dpm/mmole)	Incorporation (%)
9	^{14}C-Leu; 0.04	24	August	1.06×10^4	0.0030
10	^{14}C-Leu; 0.01	12	October	8.50×10^2	0.0002
11	^{14}C-Leu; 0.07	24	"	5.15×10^3	0.0017
12	^{14}C-Leu; 0.01	48	"	3.16×10^3	0.0008
13	^{14}C-Leu; 0.01	72	"	1.40×10^4	0.0032
14	^{14}C-Val; 0.05	24	"	2.84×10^3	0.0009
15	^{14}C-MVA; 0.02	24	August	4.76×10^3	0.0027
16	^{14}C-MVA; 0.02	24	September	1.59×10^4	0.0093

* ^{14}C-Leu, ^{14}C-Val, and ^{14}C-MVA denote L-[U-^{14}C]leucine, L-[U-^{14}C]valine, and DL-[2-^{14}C]mevalonic acid, respectively.

the tracers, these terpene alcohols were degraded by permangnate-periodate oxidation.[4] The terpene alcohol II gave levulinic acid containing the carbon atoms of C-3~C-6 and C-9 and acetone containing the carbon atoms of C-7, C-8, and C-10. The terpene alcohol III was degraded into 6-hydroxy-4-methylhexanoic acid with the carbon atoms of C-1~C-6 and C-9 and acetone with the carbon atoms of C-7, C-8, and C-10. Further purification of the acids and the acetone to constant radioactivity (Tables 3 and 4) was effected by their conversion to the methyl esters and the thiosemicarbazone derivatives, respectively. The distributions of the radioactivity in the 3,3-dimethylallyl pyrophosphate (DMAPP) moiety of geraniol (II) and citronellol (III) are based upon the C_3-fragment (isopropylidene group) representing 3/5 of the prenyl unit. The distribution in the isopentenyl pyrophosphate (IPP) moiety is, in turn, calculated by the difference, as shown in Table 5. In

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

Compounds (Carbons originated from II)	Specific radioactivity (dpm/mmole)*		
	Exp. 1	Exp. 6	Exp. 8
Geraniol (II) (C-1~C-10)	1.20×10^3	3.76×10^2	1.24×10^3
Thiosemicarbazone of acetone (C-7, C-8, and C-10)	5.30×10^2	1.90×10^2	3.08×10^2
Methyl ester of levulinic acid (C-3~C-6 and C-9)	5.60×10^2	1.86×10^2	9.33×10^2

* "Exp. No." corresponds to the number in Table 1.

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

Compounds (Carbons originated from III)	Specific radioactivity (dpm/mmole)*		
	Exp. 11	Exp. 14	Exp. 16
Citronellol (III) (C-1~C-10)	1.25×10^3	1.77×10^3	1.52×10^2
Thiosemicarbazone of acetone (C-7, C-8, and C-10)	6.20×10^2	8.36×10^2	3.08×10
Methyl ester of 6-hydroxy-4-methyl-hexanoic acid (C-1~C-6 and C-9)	6.27×10^2	9.38×10^2	1.12×10^2

* "Exp. No." corresponds to the number in Table 2.

TABLE 5. DISTRIBUTION OF RADIOACTIVITY IN IPP AND DMAPP MOIETIES

Exp. No.*	Compounds	Precursors	Distribution (%)	
			IPP	DMAPP
1	Geraniol (II)	L-[U-^{14}C]Leucine	26.2	73.8
6	"	L-[U-^{14}C]Valine	15.8	84.2
8	"	DL-[2-^{14}C]MVA	75.2	24.8
11	Citronellol (III)	L-[U-^{14}C]Leucine	17.1	82.9
14	"	L-[U-^{14}C]Valine	11.9	88.1
16	"	DL-[2-^{14}C]MVA	79.7	20.3

* "Exp. No." corresponds to the number in Tables 1 and 2.

the uptake of L-[U-^{14}C]leucine and [U-^{14}C]valine, 70~90% of the total radioactivities in geraniol (II) and citronellol (III) was located in the DMAPP moiety, whereas with DL-[2-^{14}C]MVA the predominant radioactivity was detected in the IPP moiety. The IPP-favored labeling obtained from feeding of ^{14}C-MVA has also been observed for the monoterpenes, II and III, as described in our previous paper.[5] If the monoterpene alcohols, II and III, are synthesized biologically from leucine and valine *via* MVA,

the labeling pattern should be in favor of the IPP moiety, just as the administration of MVA. These facts support that in the biosynthesis of geraniol and citronellol by the higher plant some of DMAPPs originate from leucine and valine, not *via* MVA, by some alternate route, as proposed recently for the biosynthesis of linalool (I).[1,2] It is fascinating to note that the non-mevalonoid route seems to be at least partly responsible for the unbalanced distribution of labeling in monoterpenes synthesized biologically by higher plants.[5~8]

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