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THE FIRST PROOF OF THE BIOSYNTHESIS OF ISOPRENOID FROM AMINO ACID IN HIGHER PLANT. THE INCORPORATION OF L-LEUCINE INTO LINALOOL

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U-^{14}C-L-Leucine and 4,5-^{3}H-L-leucine were incorporated into linalool (I) in *Cinnamomum Camphora* Sieb. var. *linalooliferum* Fujita in 0.004% yield at the highest value. Its labeling patterns have indicated that DMAPP may originate not only from mevalonic acid but also from leucine not via the acid.

The biosynthesis of monoterpenes is believed to involve the conversion of mevalonic acid (MVA) into isopentenyl pyrophosphate (IPP) and 3,3-dimethylallyl pyrophosphate (DMAPP), followed by the condensation of IPP with DMAPP directed toward the formation of monoterpenes. In the biosynthesis of isoprenoids from 2-^{14}C-MVA by the leaves of higher plants, the predominant location of the tracer in the moiety derived from IPP has been observed for monoterpenes,[1-4] in contrast to an equal distribution in squalene and triterpenes.[5] The unbalanced distribution of labeling in monoterpenes has been explained in terms of the operation of several factors,[1] which involve a suggestion of the presence of some other intermediates for the DMAPP biosynthesis. Since the biosynthetic pathway of steroids from leucine via MVA has been established in animal tissues,[6-9] such a pathway seems also to be probable for higher plants. However, it has been reported that labeled leucine was not incorporated[1,10] into monoterpenes by higher plant. To clarify the intermediates and pathways participating in the biosynthesis of monoterpenes, we now have tested incorporations of U-^{14}C-L-leucine and 4,5-^{3}H-L-leucine into linalool (I) by *Cinnamomum Camphora* Sieb. var. *linalooliferum* Fujita. The incorporation of 2-^{14}C-MVA has also been made for comparison with the uptake of the amino acid.

Feeding experiments were carried out on terminal branches (ca. 15 cm long) of the plant. A phosphate buffered solution (pH 7.4) of each of U-^{14}C-L-leucine, 4,5-^{3}H-L-leucine, and 2-^{14}C-DL-MVA was fed through a cut-stem into the leaves 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 (-)-linalool (I). The radioactivity of the terpene alcohol (I) was detected directly in a Packard Tri-Carb liquid scintillation spectrometer using a Bray's scintillation solvent,[11] as is shown in Table 1. These data indicate that leucine is surely incorporated into linalool (I) though a low level. From the results, linalool (I) was found to be best biosynthesized from leucine in spring and autumn,

TABLE 1. INCORPORATION OF RADIOACTIVE TRACERS INTO LINALOOL (I)

Exp. No.	Precursors* (mCi)	Feeding time (hr)	Seasons	Specific radio-activity of I (dpm/mmole)	Incorporation (%)
1	^{14}C-Leu; 0.02	24	April	1.40×10^2	0.0010
2	^{14}C-Leu; 0.025	24	May	1.70×10^2	0.0040
3	^{3}H-Leu; 0.05	12	July	3.10×10^2	0.0006
4	^{3}H-Leu; 0.05	24	"	2.77×10^2	0.0006
5	^{3}H-Leu; 0.05	36	"	3.51×10^2	0.0007
6	^{14}C-MVA; 0.04	24	"	3.15×10^3	0.0431
7	^{3}H-Leu; 0.04	12	September	9.01×10^3	0.0042
8	^{3}H-Leu; 0.04	24	"	8.45×10^3	0.0040
9	^{3}H-Leu; 0.04	36	"	5.91×10^3	0.0023
10	^{14}C-MVA; 0.02	24	"	3.05×10^3	0.0210

* ^{14}C-Leu, ^{3}H-Leu, and ^{14}C-MVA denote U-^{14}C-L-leucine, 4,5-^{3}H-L-leucine, and 2-^{14}C-DL-mevalonic acid, respectively.

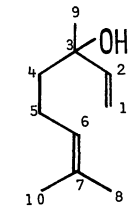
TABLE 2. DISTRIBUTION OF RADIOACTIVITIES IN LINALOOL (I) AFTER UPTAKE OF TRACERS

Compounds (Carbons originated from I)	Specific radioactivity (dpm/mmole)*			
	Exp. 1	Exp. 2	Exp. 6	Exp. 10
Linalool (I) (C-1∿C-10)	1.40×10^2	1.70×10^2	3.15×10^3	3.05×10^3
Thiosemicarbazone of acetone (C-7, C-8, and C-10)	7.01×10	8.62×10	9.50×10^2	1.05×10^3
S-Benzylthiouronium salt of lactone III (C-1∿C-6 and C-9)	7.37×10	8.40×10	2.05×10^3	1.96×10^3

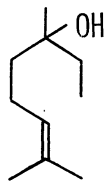
* "Exp. No." corresponds to the number in Table 1.

but lowest in summer. The same tendency has also been observed for the biosynthesis of geraniol from leucine by *Pelargonium roseum* Bourbon.[12]

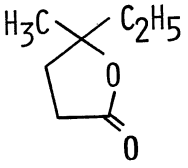
The hydrogenation of ^{14}C-labeled linalool (I) in the presence of PtO_2 gave dihydrolinalool (II), which was then degraded to 4-methyl-4-hexanolide (III) containing the carbon atoms of C-1∿C-6 and C-9 and acetone containing the carbon atoms of C-7, C-8, and C-10 by permanganate-periodate oxidation.[13] The γ-lactone (III) and the acetone were derived to the S-benzylthiouronium salt[14] and the thiosemicarbazone derivative, respectively, which were then purified to constant



I



II

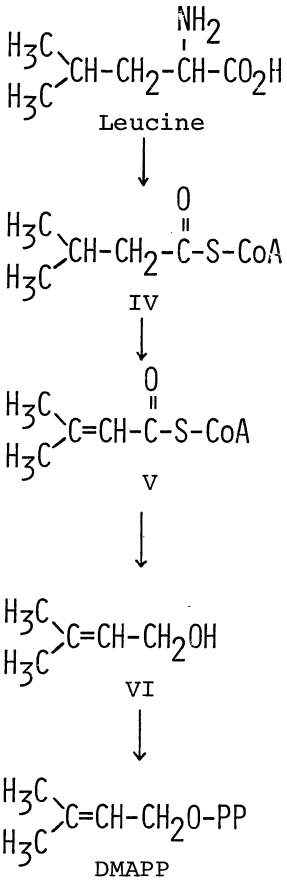


III

TABLE 3. DISTRIBUTION OF RADIOACTIVITIES IN IPP- AND DMAPP-DERIVED MOIETIES

Exp. No.*	Precursors	Distribution (%)§	
		IPP-M.	DMAPP-M.
1	^{14}C-Leu	18.7	81.3
2	^{14}C-Leu	15.6	84.4
6	^{14}C-MVA	68.3	31.7
10	^{14}C-MVA	65.1	34.9

* "Exp. No." corresponds to the number in Table 1.
§ IPP-M. and DMAPP-M. denote IPP- and DMAPP-derived moieties, respectively.



Scheme 1

specific activity upon recrystallization to determine their radioactivity (Table 2). On the basis of these labeling patterns, the distribution of the ^{14}C-tracer in the IPP- and the DMAPP-derived moieties of linalool (I) were determined by the proportional allotment, as is shown in Table 3. The DMAPP-derived moiety of linalool (I) biosynthesized from U-^{14}C-L-leucine was labeled with more than 80% of the incorporated tracer, whereas the moiety of the alcohol (I) biosynthesized from 2-^{14}C-MVA contained less than 35% of the tracer. If linalool (I) is biosynthesized from leucine via MVA, the labeling pattern should be similar to the pattern in the alcohol (I) biosynthesized from 2-^{14}C-MVA. These facts indicate that some of DMAPPs may originate from leucine, not via MVA, by some alternate route.

This is the first proof of the biosynthesis of monoterpene by a non-mevalonoid route. We now wish to propose a pathway in which the DMAPP may be derived from leucine, as is shown in Scheme 1. Transamination, followed by decarboxylation, of leucine produces isovaleric acid, which is in an activated form (IV) and is then converted into dimethylacrylyl CoA (V) by dehydrogenation, as has been proved for the metabolism of leucine in animal tissues.[6-9] Reduction of V with NADPH or NADH affords 3,3-dimethylallyl alcohol (VI), which gives rise to DMAPP. As cited above, the biosynthesis of

monoterpenes should be rationalized in the concerted operation of several factors. However, any factor has not been clarified yet. The present establishment of the incorporation of the amino acid into the monoterpene through a non-mevalonoid route now has given a clue to an elucidation of the complex factors participating in the biosynthesis of monoterpenes by higher plants.

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References

1) D. V. Banthorpe, B. V. Charlwood, and M. J. O. Francis, Chem. Rev., 72, 115 (1972) and the related papers cited therein.
2) T. Suga, T. Shishibori, K. Kotera, and R. Fujii, This Letter, 1972, 533.
3) T. Suga and T. Shishibori, ibid., 1972, 1093.
4) D. V. Banthorpe and G. N. J. Le Patourel, Biochem. J., 130, 1055 (1972).
5) R. Croteau and W. D. Loomis, Phytochemistry, 12, 1957 (1973) and the related papers cited therein.
6) M. J. Coon, J. Biol. Chem., 187, 71 (1950).
7) F. Lynen, Proc. Intern. Symp. Enzyme Chem., Tokyo-Kyoto, 1957, 57.
8) A. del Campillo-Campbell, E. E. Dekker, and M. J. Coon, Biochim. Biophys. Acta, 31, 290 (1959).
9) H. Hily, J. Knappe, E. Ringelmann, and F. Lynen, Biochem. Z., 329, 476 (1958).
10) W. D. Loomis, "Terpenoids in Plants," J. B. Pridham, Ed., Academic Press, London and New York (1967).
11) G. A. Bray, Analyt. Biochem., 1, 279 (1960).
12) T. Suga, T. Hirata, and K. Tange, unpublished data.
13) T. Suga and E. von Rudloff, J. Sci. Hiroshima Univ., A-II, 34, 69 (1970).
14) C.-T. Chang, Formosan Sci., 16, 127 (1962).

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