CHEMISTRY LETTERS, pp. 777-780, 1981.

THE CORROBORATION OF THE PREDOMINANT LOCALIZATION OF RADIOACTIVITY ON THE DIMETHYLALLYL PYROPHOSPHATE-DERIVED MOIETY OF LINALOOL BIOSYNTHESIZED FROM RADIOISOTOPICALLY LABELED LEUCINE BY HIGHER PLANTS

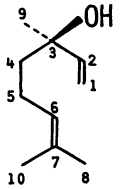
Keiji TANGE, Hitoshi OKITA, Yoshitaka NAKAO, Toshifumi HIRATA, and Takayuki SUGA*

Department of Chemistry, Faculty of Science, Hiroshima University, Higashisenda-machi, Naka-ku, Hiroshima 730

The co-feeding experiment of leucine-4,5-^{3}H and mevalonic-2-^{14}C acid corroborated the preferential localization of radioactivity on the 3,3-dimethylallyl pyrophosphate-derived moiety of linalool in its biosynthesis from radioisotopically labeled leucine by *Cinnamomum Camphora* Sieb. var. *linalooliferum* Fujita, in contrast to the predominant location of the activity on its isopentenyl pyrophosphate-derived moiety in the biosynthesis from mevalonic acid. Also, it was established that the imbalance in the localization of radioactivity is not influenced by exogenous administration of leucine or inhibition of isopentenyl pyrophosphate isomerase.

Monoterpenoids biosynthesized from radioisotopically labeled mevalonic acid (MVA) by higher plants have shown the predominant localization of radioactivity on their isopentenyl pyrophosphate(IPP)-derived moiety.[1-4] This also has been the case for linalool (I) biosynthesized from radioisotopically labeled alanine.[5] Such an asymmetric localization of radioactivity has been explained in terms of operation of several factors;[3] one of which is that the predominant localization may result from the inhibition of IPP isomerase by exogenously administered MVA or biologically pooled MVA. In contrast, the predominant localization of radioactivity on the 3,3-dimethylallyl pyrophosphate(DMAPP)-derived moiety was observed for monoterpenoids biosynthesized from radioisotopically labeled leucine and valine.[6-9]

In order to corroborate the contrasted imbalance in the distribution of radioactivity, we examined (i) the distribution of radioactivity in the IPP- and DMAPP-derived moieties of linalool (I) biosynthesized by co-feeding of ^{3}H-labeled leucine and ^{14}C-labeled MVA, (ii) the influence of exogenous administration of these precursors on the distribution of radioactivity in the IPP- and DMAPP-derived moieties,



I

IPP-derived moiety

DMAPP-derived moiety

Table 1. $^{3}H/^{14}C$-Ratios in linalool (I) and its IPP- and DMAPP-derived moieties after co-feeding of DL-leucine-4,5-^{3}H and DL-MVA-2-^{14}C

Biosynthetic product and its moieties	Sp. radioact. ^{3}H (dpm/mmol)	Sp. radioact. ^{14}C (dpm/mmol)	$^{3}H/^{14}C$	$^{3}H/^{14}C$ normalized to linalool (I)
Linalool (I)	2.18×10^{3}	2.85×10^{3}	0.76	1
IPP-M.[a]	7.40×10^{2}	1.66×10^{3}	0.45	0.59[b]
DMAPP-M.[a]	1.43×10^{3}	1.21×10^{3}	1.18	1.55[b]

a) IPP-M. and DMAPP-M. denote IPP- and DMAPP-derived moieties, respectively.
b) The deviation for the values is about ±0.05.

and (iii) the influence of inhibition of IPP isomerase on the distribution of radioactivity in the IPP- and DMAPP-derived moieties of I biosynthesized from ^{14}C-labeled leucine.

A mixture of DL-leucine-4,5-^{3}H (50 μCi) and DL-MVA-2-^{14}C (5 μCi) was fed to the sprigs of *Cinnamomum Camphora* Sieb. var. *linalooliferum* Fujita weighed ca. 10 g in fresh weight in the same manner as described in our previous communication.[6] Radioactive linalool (I) isolated from the sprigs was subjected to the oxidative degradation with permanganate-periodate reagent[10] in the same procedure as described in our previous reports[6-9] to give degradation products, such as acetone, formaldehyde, and levulinic acid. These products were converted to acetone thiosemicarbazone, formaldehyde bisdimedone, and methyl levulinate thiosemicarbazone derivatives, which were purified to a constant specific radioactivity by repeated recrystallization. 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 of linalool (I).[6,9] On the other hand, the tracer originating from MVA-2-^{14}C would reside on the C-4 and the C-8 carbon atoms of I.[9,11] In the incorporation of the mixture of ^{3}H-labeled leucine and ^{14}C-labeled MVA, therefore, all the radioactivities located on the IPP- and DMAPP-derived moieties would appear in molecules of levulinic acid and acetone, respectively. $^{3}H/^{14}C$-Ratios in these moieties thus obtained are shown in Table 1. When the $^{3}H/^{14}C$-ratio in linalool (I) is normalized to 1, the ratios in the IPP- and DMAPP-derived moieties are 0.59 and 1.55, respectively. This indicates that the distribution of ^{3}H originating from leucine is greater in the DMAPP-derived moiety than in the IPP-derived one, whereas the distribution of ^{14}C from MVA reverses between these moieties. Thus, co-feeding of ^{3}H-labeled leucine and ^{14}C-labeled MVA has now corroborated the contrasted imbalance in the distribution of the radioactivity originating from these precursors between the IPP- and DMAPP-derived moieties of the monoterpenoids[1-4, 6-9] biosynthesized from leucine and MVA each.

Next, the influence of exogenous administration of the precursors on the distribution of radioactivity in the IPP- and DMAPP-derived moieties was examined by feeding of varied amounts of leucine and MVA, as shown in Table 2. L-Leucine-U-^{14}C and DL-MVA-2-^{14}C diluted with the non-radioactive sample were separately administered to the sprigs in the same manner as above. The results shown in Table 2 indicate that not only the incorporation of these precursors into linalool (I), but the imbalance in the distribution of radioactivity in the IPP- and DMAPP-derived moieties

Table 2. The influence of exogenous administration of L-leucine-U-^{14}C and DL-MVA-2-^{14}C on the distribution of radioactivity in the IPP- and DMAPP-derived moieties of linalool (I)

Precursors			Linalool (I) biosynthesized		Distribution of radioact. (%)[b]	
Compd.	Radioact. (μCi)	Amount administered (mg)	Sp. radioact. (dpm/mmole)	Incorp.[a] (%)	IPP-M.[c]	DMAPP-M.[c]
Leu-U-^{14}C	40	0.27	1.09×10^3	0.00043	31	69
"	10	1.3	1.25×10^3	0.0028	—	—
"	40	2.5	2.16×10^3	0.00096	38	62
"	10	3.8	6.66×10^2	0.0025	—	—
"	40	5.0	4.40×10^3	0.0055	39	61
MVA-2-^{14}C	4	0.15	2.85×10^3	0.023	63	37
"	4	1.1	4.95×10^3	0.047	58	42
"	4	5.1	3.46×10^3	0.027	—	—
"	4	10.3	2.28×10^3	0.022	—	—
"	4	15.1	1.38×10^3	0.013	—	—
"	4	20.0	1.13×10^3	0.011	58	42

a) Calculated as the only 3*R*-enantiomer of a racemic mixture of MVA participates in the formation of I.
b) The deviation for the values is about ±2%.
c) Refer to a) in Table 1.

is not influenced by change in the quantity of the exogenously administered precursors. Thus, it has been found that exogenous administration of leucine and MVA is not responsible for the asymmetric localization of radioactivity.

We finally tested the distribution of radioactivity in the IPP- and DMAPP-derived moieties of I biosynthesized from leucine-4,5-^{3}H and MVA-5-^{3}H by the plant which had been pre-treated with an IPP isomerase inhibitor. Iodoacetamide (IAA) was used as the inhibitor.[12-14] DL-leucine-4,5-^{3}H and DL-MVA-5-^{3}H were administered to the

Table 3. The influence of iodoacetamide on the incorporation of DL-leucine-4,5-^{3}H and DL-MVA-5-^{3}H into linalool (I) and the asymmetric distribution of radioactivity in its IPP- and DMAPP-derived moieties

Precursors (μCi)	Inhibitor (IAA)	Linalool (I) biosynthesized		Distribution of radioact. (%)[b]	
		Sp. radioact. (dpm/mmole)	Incorp.[a] (%)	IPP-M.[c]	DMAPP-M.[c]
Leu-4,5-^{3}H (50)	None	1.01×10^3	0.0012	32	68
" (50)	Added	1.85×10^3	0.0017	29	71
MVA-5-^{3}H (10)	None	8.98×10^3	0.076	59	41
" (10)	Added	1.06×10^3	0.0070	79	21

a) Calculated with respect to the only L- and 3*R*-enantiomer of the precursors similar to a) in Table 2.
b, c) Refer to b) in Table 2 and a) in Table 1, respectively.

sprigs (ca. 20 g) just after soak up of 0.01 mol·dm^{-3} IAA solution for 3 h. The results are shown in Table 3. It was found that pre-treatment of the plant with IAA does not influence the incorporation of the precursors as well as the predominant localization of radioactivity on the DMAPP portion in the biosynthesis of linalool (I) from leucine-4,5-^{3}H, but it results in a decrease in the incorporation of the precursors and an increase in the imbalance in the localization of radioactivity in its biosynthesis from MVA-5-^{3}H.

It now has been established that the imbalance in the distribution of radioactivity observed in the biosynthesis of linalool (I) from MVA is reversed in its biosynthesis from leucine and also the asymmetric distribution of radioactivity is not ascribed to exogenous administration of the precursors. If linalool (I) is biosynthesized from leucine *via* MVA, the distribution pattern would be similar to the pattern in I biosynthesized from MVA. Accordingly, it is considered that leucine is incorporated into I, not *via* MVA, by an alternate route.[6-9] The participation of the route in the biosynthesis of I from leucine is supported by the fact that the IPP isomerase inhibitor did not influence the distribution of radioactivity in the IPP- and DMAPP-portions, in contrast to the biosynthesis from MVA. Thus, it is fascinating to note that the amino acids having a carbon-skeleton similar to MVA may participate in the biosynthesis of monoterpenoids by their direct conversion to DMAPP through the alternate route rather than the mevalonate pathway.

Acknowledgments. The authors thank Dr. Tsuyoshi SHISHIBORI for discussion. The present work was partially supported by a Grant-in-Aid for Scientific Research No. 347024 (1980, to T.S.) from the Ministry of Education, Science and Culture.

References

* To whom all inquiries should be addressed.
1) T. Suga, T. Shishibori, K. Kotera, and R. Fujii, Chem. Lett., 1972, 533.
2) T. Suga and T. Shishibori, Bull. Chem. Soc. Jpn., 46, 3545 (1973).
3) D. V. Banthorpe, B. V. Charlwood, and M. J. O. Francis, Chem. Rev., 72, 115 (1972) and the related papers cited therein.
4) D. V. Banthorpe and G. N. J. Le Patourel, Biochem. J., 130, 1055 (1972).
5) K. Tange, T. Hirata, and T. Suga, Chem. Lett., 1979, 269.
6) T. Suga, T. Hirata, T. Shishibori, and K. Tange, Chem. Lett., 1974, 189.
7) T. Suga, T. Hirata, and K. Tange, Chem. Lett., 1975, 131.
8) T. Suga, T. Hirata, and K. Tange, Chem. Lett., 1975, 243.
9) K. Tange, Bull. Chem. Soc. Jpn., submitted for publication.
10) T. Suga and E. von Rudloff, J. Sci. Hiroshima Univ., A-II, 34, 69 (1970).
11) T. Suga, T. Shishibori, and M. Bukeo, Bull. Chem. Soc. Jpn., 45, 1480 (1972).
12) C. R. Benedict, J. Kett, and J. W. Porter, Arch. Biochem. Biophys., 110, 611 (1965).
13) K. Ogura, T. Nishino, and S. Seto, J. Biochem. (Tokyo), 64, 197 (1968).
14) D. H. Shah, W. W. Cleland, and J. W. Porter, J. Biol. Chem., 240, 1946 (1965).

(Received April 11, 1981)