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BIOSYNTHESIS OF CYCLIC MONOTERPENES IN HIGHER PLANTS. THE RETENTION OF THE C-5 HYDROGEN ATOMS OF MEVALONATE DURING ITS INCORPORATION INTO THE CYCLIC MONOTERPENES

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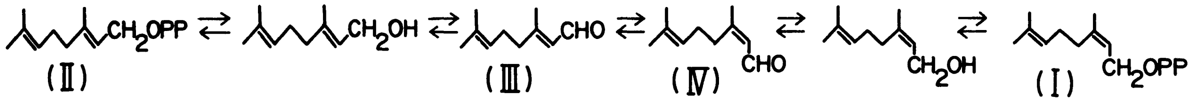
The $^{3}H/^{14}C$ ratio of [5-$^{3}H_{2}$, 2-^{14}C]mevalonate was preserved in limonene, perillaldehyde, and camphor biosynthesized by higher plants from the mevalonate. This indicates that the cyclic monoterpenes are biosynthesized through such a pathway that involves the cyclization of such an intermediate as retaining all the C-1 and C-5 tritium atoms of geranyl pyrophosphate derived from the mevalonate.

In general, the biosynthesis of cyclic monoterpenes is said to involve the cyclization of neryl pyrophosphate (NPP) (I). The most likely one of several mechanisms1~3) proposed for the biosynthesis of NPP is the isomerization of geranyl pyrophosphate (GPP) (II) to NPP *via* the corresponding aldehyde intermediates (III) and (IV)2,4~7) (Scheme 1). On the other hand, the possible intermediacy of linaloyl pyrophosphate in the isomerization is inferred from analogy with the proposal8) that 2,6-*trans,trans*-farnesyl pyrophosphate is isomerized to 2,6-*cis, trans*-farnesyl pyrophosphate *via* nerolidyl pyrophosphate. We now have tested whether the aldehyde intermediate or other one is involved in the isomerization of GPP to NPP that probably occurs during the formation of cyclic monoterpenes from GPP derived from mevalonate in higher plants.

[5-$^{3}H_{2}$, 2-^{14}C]Mevalonic acid ($^{3}H/^{14}C$ = 3.31, 10 μCi of ^{14}C) dissolved in a phosphate buffered solution (pH 7.4) was administered to the cut-peels of the fruits of *Citrus Natsudaidai* Hayata and to the leaves of *Perilla frutescens* Britton and *Cinnamomum Camphora* Sieb. through their cut-stems. The plant materials, after one day, were extracted with *n*-hexane to give the hexane extracts, which then on chromatographic separation by means of preparative TLC afforded (+)-limonene (V), (−)-perillaldehyde (VI), and (+)-camphor (VII), respectively. Compounds V and VI were converted to *p*-menth-8-ene-*trans*-1,2-diol and perillyl alcohol, respectively, while VII was subjected to sublimation as such. These compounds were purified to a constant specific activity to determine their radioactivities and then the $^{3}H/^{14}C$ ratios and the $^{3}H/^{14}C$ atom ratios in V, VI, and VII, as shown in Table 1. The initial $^{3}H/^{14}C$ ratio in mevalonate was preserved in V, VI, and VII biosynthesized by



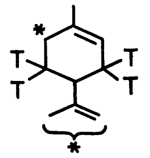
Scheme 1.

TABLE 1. $^{3}H/^{14}C$ ATOM RATIOS IN THE CYCLIC MONOTERPENES BIOSYNTHESIZED FROM $[5-^{3}H_2, 2-^{14}C]$MEVALONATE BY SUCH PLANT MATERIALS AS PRODUCING MONOTERPENES

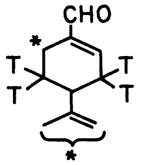
Compounds	Observed				Expected*	
	^{3}H (dpm)	^{14}C (dpm)	$^{3}H/^{14}C$ ratio	Atom ratio (^{3}H : ^{14}C)	*via* Route A (^{3}H : ^{14}C)	*via* Route B (^{3}H : ^{14}C)
Limonene (V)	1616	478	3.38	4.08 : 2	3 : 2	4 : 2
Perillaldehyde (VI)	3633	1090	3.33	4.02 : 2	3 : 2	4 : 2
Camphor (VII)	2720	860	3.16	3.81 : 2	3 : 2	4 : 2

* The expected $^{3}H/^{14}C$ atom ratios were calculated by assuming that V, VI, and VII are formed by cyclization of I produced *via* either the aldehyde intermediates, (III) and (IV), which accordingly lose one of the C-1 hydrogen atoms of II (route A), or other intermediate which retains both of the C-1 hydrogen atoms (route B).

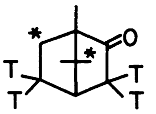
uptake of the mevalonate. This indicates that V, VI, and VII were biosynthesized without the loss of any proton of GPP derived from mevalonate, and labeled as illustrated in formulae V, VI, and VII. If the isomerization of GPP to NPP that probably occurs during the formation of cyclic monoterpenes proceeds *via* such aldehyde intermediates as geranial (III) and neral (IV) as shown in Scheme 1,[5~7] the $^{3}H/^{14}C$ atom ratios in the cyclic monoterpenes (V), (VI), and (VII) resulted from the cyclization of NPP should be 3 to 2 as a result of the loss of one of the C-1 tritium atoms in GPP derived from the doubly labeled mevalonate. On the other hand, if the isomerization proceeds without the loss of any tritium atom, the atom ratio should be 4 to 2. The observed $^{3}H/^{14}C$ atom ratios in the cyclic monoterpenes were consistent with the ratio expected for the latter case, as shown in Table 1.



(V)



(VI)



(VII)

The present feeding experiments indicated that in the plant materials used in these experiments the cyclic monoterpenes were biosynthesized through such a pathway that involves the cyclization of an intermediate, which is produced by the isomerization of GPP and retains all of its C-1 and C-5 hydrogen atoms originated from C-5 of mevalonate. It was thus found that the *cis,trans*-isomerization of GPP to NPP that probably occurs during the formation of cyclic monoterpenes in higher plants does proceed *via* such an intermediate, but not such corresponding aldehyde intermediates as (III) and (IV), as retaining both of the C-1 hydrogen atoms of GPP. This is in contrast with the isomerization of 2,6-*trans,trans*-farnesol to 2,6-*cis, trans*-farnesol *via* the corresponding aldehyde intermediate, farnesal, in microorganisms.[5,6]

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