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RETENTION OF *pro*-4*R* HYDROGEN ATOM OF MEVALONIC ACID IN BIOSYNTHESIS OF CYCLIC MONOTERPENOIDS. EVIDENCE FOR INDIRECT FORMATION OF *cis*-ISOPRENE UNIT FROM ISOPENTENYL AND DIMETHYLALLYL PYROPHOSPHATES

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The *pro*-4*R* hydrogen atom of mevalonic acid was preserved in perillaldehyde, carvone, menthol, and limonene biosynthesized from the mevalonic acid by higher plants. This is evidence that such a condensation of isopentenyl pyrophosphate with dimethylallyl pyrophosphate as directly giving rise to a *cis*-isoprene unit is not involved in the biosynthesis of cyclic monoterpenoids in higher plants.

It is generally accepted that the biosynthesis of cyclic monoterpenoids involves the cyclization of neryl pyrophosphate (NPP) or its biogenetic equivalent with a *cis* ethylenic linkage favorable to the cyclization.[1,2] The most likely mechanism for the formation of NPP appears to involve either such a direct condensation of isopentenyl pyrophosphate (IPP) with 3,3-dimethylallyl pyrophosphate (DMAPP) as giving rise to a *cis*-isoprene unit, or the initial formation of geranyl pyrophosphate (GPP) followed by its isomerization to NPP.[3] We now have tested whether the direct *cis* condensation of IPP with DMAPP is involved or not in the biosynthesis of cyclic monoterpenoids from mevalonic acid by higher plants.

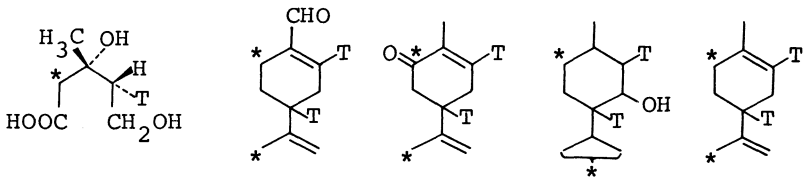
Potassium salt of mevalonic acid-2-^{14}C, (4*R*)-4-^{3}H (I) (10 μCi of ^{14}C) dissolved in a phosphate-buffered solution (pH 7.4) was administered, for a day, to the leaves of *Perilla frutescens* Britton, *Mentha spicata* L., and *Mentha piperita* L. through their cut-stems and to the cut-peels of the fruits of *Citrus Natsudaidai* Hayata, separately. The plant materials were extracted with hexane to give a hexane extract, which upon chromatographic separation by means of preparative TLC afforded (-)-perillaldehyde (II), (-)-carvone (III), (-)-menthol (IV), and (+)-limonene (V) as a main component, respectively. II was converted to perillyl alcohol, III to the semicarbazone derivative, IV to its 3,5-dinitrobenzoate, and V to *p*-menth-8-ene-1,2-diol *via* *p*-menth-8-en-1,2-oxide, respectively. These compounds were purified to a constant specific activity to determine their radioactivities, the ^{3}H/^{14}C ratios, and the ^{3}H/^{14}C atom ratios, which are shown in Table 1. The initial ^{3}H/^{14}C ratio in mevalonic acid was preserved in II, III, IV, and V. This indicates that II, III, IV, and V were formed biologically without loss of the 4*R*-tritium atom of mevalonic acid (I), and the cyclic monoterpenoids (II)-(V) are predicted to be labeled as illustrated in the following formulae.[4,5]

It has been shown that the formation of double-bonds in all-*trans* isoprenoids involves loss of the *pro*-4*S* hydrogen atom of mevalonic acid,[6] while *cis*-isoprene

TABLE 1. ISOTOPE RATIOS IN THE CYCLIC MONOTERPENOIDS BIOSYNTHESIZED FROM MEVALONIC ACID-2-^{14}C, (4*R*)-4-^{3}H

Monoterpenoids	^{3}H/^{14}C in MVA used	Observed				Expected*	
		^{3}H (dpm)	^{14}C (dpm)	^{3}H/^{14}C	Atom ratio (^{3}H : ^{14}C)	Route A (^{3}H : ^{14}C)	Route B (^{3}H : ^{14}C)
Perillaldehye (II)	4.28	1801	383	4.70	2.20 : 2	1 : 2	2 : 2
Carvone (III)	4.28	547	132	4.14	1.93 : 2	1 : 2	2 : 2
Menthol (IV)	7.56	5302	632	8.39	2.22 : 2	1 : 2	2 : 2
Limonene (V)	7.56	1484	211	7.03	1.86 : 2	1 : 2	2 : 2

* The expected atom ratios were calculated by assuming that the monoterpenoids (II)-(V) are biosynthesized through either a route A or B, as described in the text.



I II III IV V

* and T denote ^{14}C and ^{3}H, respectively.

residues of natural rubber arise from elimination of the epimeric *pro*-4*R* atom.[7] If NPP is formed directly by *cis* condensation of IPP with DMAPP and followed by cyclization to cyclic monoterpenoids (route A), the atom ratio of 1 to 2 is expected as a result of loss of the 4*R*-tritium atom of mevalonic acid (I) (Table 1). On the other hand, if GPP is produced first by *trans* condensation of IPP with DMAPP and gives rise to cyclic monoterpenoids through its isomerization to NPP followed by cyclization (route B), the atom ratio should be 2 to 2 as a result of retention of the 4*R*-tritium atom (Table 1). The ^{3}H/^{14}C atom ratios observed for the cyclic monoterpenoids (II)-(V) were consistent with the ratio expected for the latter case, *i.e.*, retention of the 4*R*-tritium atom, as shown in Table 1.

The result of the tracer experiment indicates unambigously that such a condensation of IPP with DMAPP as directly giving rise to a *cis*-isoprene unit is not involved in the biosynthesis of cyclic monoterpenoids in higher plants. Thus, the cyclic monoterpenoids are considered to be biosynthesized in the following sequence: the first formation of GPP by *trans* condensation of IPP with DMAPP, followed by isomerization of GPP to a proximate intermediate or NPP through such a pathway as retaining both the C-1 hydrogen atoms of GPP[8] and the cyclization of the intermediate or NPP to cyclic monoterpenoids.

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