Preferential Participation of Linaloyl Pyrophosphate rather than Neryl Pyrophosphate in Biosynthesis of Cyclic Monoterpenoids in Higher Plants

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Summary Comparisons of the incorporation of [1-$^3H_2$]-nerol [1-$^3H_2$]geraniol, and [1-$^3H_2$]linalool and their pyrophosphates into some cyclic monoterpenoids have been made in both intact plants and cell-free systems, the preferential incorporation of linalool and its pyrophosphate into the cyclic monoterpenoids suggests that they are better precursors than nerol or its pyrophosphate for the biological formation of the cyclic monoterpenoids

BIOSYNTHESIS of cyclic monoterpenoids is generally assumed to involve the cyclization of neryl pyrophosphate (NPP) which contains a *cis* ethylenic linkage which favours cyclization [1,2] In the biosynthesis of the cyclic monoterpenoids, geranyl pyrophosphate (GPP) is formed first[3–5] from mevalonic acid and may be transformed into NPP *via trans–cis* isomerization Retention of the C-5 hydrogen atoms of mevalonic acid in the cyclic monoterpenoids ruled out the involvement of an aldehyde intermediate in this isomerization [6,7] Participation of nerolidyl pyrophosphate in the isomerization of farnesyl pyrophosphate has been proposed,[8,9] and recently, the intermediacy of nerolidyl pyrophosphate in the biosynthesis of cyclonerodiol has been demonstrated in a fungus [10] We have now examined the participation of linalool and its pyrophosphate (LPP) in the biological formation of cyclic monoterpenoids in higher plants

Retention of the C-1 hydrogen atoms of NPP GPP, and LPP during their transformation to cyclic monoterpenoids was proved by incubating [1-^{14}C, $^3H_2$]NPP (^{3}H/^{14}C ratio, 5 32) GPP (4 53) and LPP (10 7) with a cell-free system prepared from growing tips of *Mentha spicata* L according to the described procedure [11] The product contained α-terpineol as major component but the product obtained from the boiled-enzyme control contained only the acyclic terpene alcohol corresponding to the pyrophosphate used The ^{3}H/^{14}C ratios in the α-terpineol formed from NPP, GPP, and LPP were 5 30 4 00 and 11 6, respectively the same as in the starting pyrophosphates This indicates retention of the C-1 tritium atoms during the biological formation of α terpineol from all the pyrophosphates and agrees with the result obtained from the administration of [5-$^3H_2$]-mevalonic acid [6] Accordingly, LPP, but not an aldehyde intermediate,[12] is suggested to participate in the *trans-cis* isomerization of GPP as well as in the biological formation of cyclic monoterpenoids in higher plants

The incorporation of [1-$^3H_2$]LPP into α-terpineol and carvone was compared with those of [1 $^3H_2$]NPP and [1-$^3H_2$]GPP in the cell-free system The result shown in the Table indicates that LPP is incorporated into α-terpineol and carvone in preference to NPP This suggests the direct formation of α-terpineol from LPP with the enzyme system, although there is no doubt about the derivation of α-terpineol from NPP

TABLE Incorporation of [1 $^3H_2$]NPP [1 $^3H_2$]GPP and [1 $^3H_2$]LPP into α terpineol and carvone in a cell free system from *Mentha spicata*

Substrate	(d p m)	α-Terpineol		Carvone	
		D p m	Incorpn %	D p m	Incorpn %
NPP	(1 48 × 10^6)	5 12 × 10^4	3 46	8 98 × 10^2	0 061
GPP	(6 46 × 10^6)	6 92 × 10^4	1 08	7 40 × 10^2	0 012
LPP	(6 22 × 10^5)	3 74 × 10^4	12 0	3 14 × 10^2	0 101

The preferential incorporation of linalool and LPP into (+)-limonene and (−)-perillaldehyde was further supported by feeding experiments using the intact peels of fruits of *Citrus Natsudaidai* Hayata and the leaves of *Perilla frutescens* Britton *var acta* Kudo

The incoporation of linalool was directly compared with those of nerol and geraniol by administering pairs of substrates such as [1 $^3H_2$]nerol and [1-^{14}C]linalool ($^3H/^{14}C$ 19 6) and [1-$^3H_2$]geraniol and [1-^{14}C]linalool (23 1) to the leaves of *M spicata* by the cut-stem method The $^3H/^{14}C$ ratios of the carvone isolated were 12 7 and 2 5 for the respective incorporation of these pairs of substrates indicating clearly that the incorporation of linalool into carvone predominates over those of nerol and geraniol

All these results suggest that linalool or LPP is a better precursor than nerol or NPP for the biological formation of the cyclic monoterpenoids in the higher plants investigated

The present work was partially supported by a Grant in-Aid for Scientific Research from the Ministry of Education, Science and Culture and scholarships from the Saneyoshi Scholarship Foundation and the Kudo Science Foundation

(*Received* 14*th September* 1979, *Com* 984)

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