Biosynthesis of 2-*trans*,6-*trans*- and 2-*cis*,6-*trans*-Farnesols by Soluble Enzymes from Tissue Cultures of *Andrographis paniculata*

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Summary A cell-free system from *Andrographis paniculata* tissue cultures incorporates ^3H from (3*RS*)-[2-^{14}C,-5-$^3H_2$]mevalonate into *trans,trans*-farnesol without loss and into the *cis,trans*-isomer with loss of one sixth of the label; this strongly supports *trans* → *cis* isomerisation *via* aldehydes.

NATURAL isoprenoids contain both *trans* and *cis* substituted double bonds. It has been generally accepted[1] that there is a specific correlation in isoprenoid biosynthesis between the geometry of a double bond and the configuration of the prochiral C-4 hydrogen atom of mevalonic acid lost in its formation. Specifically, isoprenoids that lose the 4-*pro-S* hydrogen atom are held to be biogenetically *trans*, while those that lose the 4-*pro-R* hydrogen atom are biogenetically *cis*.

Recent results have made it necessary to question these assumptions. Thus it has been shown that the terminal *cis* double bonds of nerol,[2] 2-*cis*,6-*trans*-farnesol,[3] and abscisic acid[4] are all formed with loss of the 4-*pro-S* and retention of the 4-*pro-R* hydrogen atom of mevalonic acid, in conflict with previously held views. Two suggestions have been made to account for these new findings: (i) a biogenetically transoid double bond is formed initially by the accepted mechanism and subsequently isomerised to a cisoid double bond;[2,5] *or* (ii) *cis* double bonds can be formed directly in one of two stereochemically distinct ways, utilizing either two different enzymes or one enzyme with two alternative binding sites.[2,3]

TABLE

(3*RS*)-[2-^{14}C,5-$^3H_2$]mevalonate ..	10·64	9·31
trans,trans-OAc	10·46	9·04
trans,trans-OSiMe$_3$	10·52	9·06
cis,trans-OAc	8·43	7·56
cis,trans-OSiMe$_3$	8·52	7·63
Calc. for 1/6 ^3H loss	8·86	7·77

We have obtained evidence that strongly supports isomerisation of 2,6-*trans,trans*- to 2-*cis*,6-*trans*-farnesol *via* aldehyde intermediates. A cell-free system was prepared, by centrifugation at 105,000 g, from callus tissues of *Andrographis paniculata* grown in suspension culture. It incorporated 10% of the radioactivity from (3*R*)-[2-^{14}C]-

mevalonate into 2-*trans*,6-*trans*- and 2-*cis*,6-*trans*-farnesols in the proportion 5 : 1 (radio-g.l.c. and t.l.c. of alcohols, acetates, and trimethylsilyl ethers). There was total loss of ^3H label from (3*RS*)-[2-^{14}C,4*S*-4-$^3H_1$]mevalonate and total retention from the (4*R*)-isomer in *both* the *trans*,*trans*- and *cis*,*trans*-farnesols formed, in agreement with previous findings.[3] With (3*RS*)-[2-^{14}C,5-$^3H_2$]mevalonate, there was total retention of ^3H in the *trans*,*trans*- but loss of one sixth of the ^3H label in the *cis*,*trans*-isomer, as shown by the ^3H : ^{14}C ratios in duplicate experiments (see Table).

These results support *trans* → *cis* isomerisation *via* aldehydic intermediates and make it unnecessary in this case to invoke intervention of a prenyl transferase different from that responsible for *cis* double bond formation in rubber[6] and polyprenol[1,7] biosynthesis.

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