Biosynthesis of 2-*cis*-Farnesol

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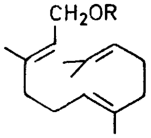
Summary 2-*cis*-Farnesol and trichodiene are formed by a cell-free preparation from *Trichothecium roseum*, from all-*trans*-farnesyl pyrophosphate with the unexpected loss of a 5,5-$^3H_2$-mevalonoid tritium label.

The cyclization of farnesyl pyrophosphate to form many sesquiterpenoids, including those of the trichothecane class, requires the 2-*cis*-isomer.[1] [1-^{14}C]Farnesyl pyrophosphate, prepared chemically as a mixture of C-2 double-bond isomers, has been shown[2] to be a precursor of trichothecin (**4**) in *Trichothecium roseum.* A 4-*pro-R* mevalonoid hydrogen label is retained[3] on the C-2 double bond which becomes C-10 of trichothecin.

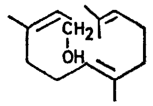
Table. *Incubation of all*-trans-[1,1,5,5,9,9-$^3H_6$;4,8,12-$^{14}C_3$]-*farnesyl pyrophosphate*

	^{14}C d.p.m.	^{3}H d.p.m.	^{3}H : ^{14}C	Atoms ^{3}H
Farnesyl pyrophosphate ..	2,366,784	17,391,666	7·35 : 1	6
all-*trans*-Farnesol	49,480	361,204	7·30 : 1	5·96
2-*cis*-6-*trans*-Farnesol	9740	60,390	6·20 : 1	5·06
Trichodiene ..	127,300	737,300	5·95 : 1	4·86

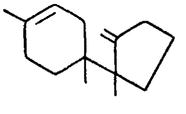
A cell-free system mediating the cyclization of all-*trans*-farnesyl pyrophosphate (**1**; R = $P_2O_6H_3$) to trichodiene (**3**) has been prepared from *Trichothecium roseum.* The mycelium from a 3-day old culture was disrupted in a French press. The supernatant liquid from a 10,000 g centrifugation was then subjected to a 1% protamine sulphate precipitation followed by a second centrifugation. all-*trans*-[1,1,5,5,9,9-$^3H_6$;4,8,12-$^{14}C_3$]Farnesyl pyrophosphate was prepared enzymatically from [5,5-$^3H_2$;2-^{14}C]mevalonate using a fresh pig-liver system. It was checked for isomeric purity by hydrolysis with alkaline phosphatase. The all-*trans*-farnesyl pyrophosphate was incubated with the cell-free system at pH 7·0 in the presence of NADP, NADH, and $MgCl_2$. The products were extracted with ethyl acetate and purified by repeated t.l.c. first on silica plates then on silica–silver nitrate plates. all-*trans*-Farnesol (**1**; R = H), 2-*cis*-6-*trans*-farnesol (**2**), trichodiene (**3**), and a hitherto unidentified hydrocarbon were isolated. Examination of the farnesal fraction revealed a small incorporation into these substances. The hydrocarbon trichodiene was converted into trichothecin (**4**) in 5% yield by an intact culture of *Trichothecium roseum.*[4] These results are in the Table.



(1)



(2)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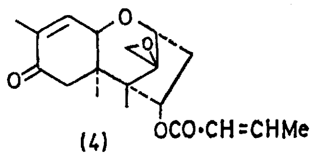

(4)

The ^{3}H : ^{14}C ratio in the 2-*cis*-farnesol and the trichodiene shows that there is an unexpected loss of one hydrogen atom in their formation from all-*trans*-farnesyl pyrophosphate, which, taking into account our earlier work on trichothecin,[3] is from C-1. Very recently a time-course study with an enzyme preparation from orange flavedo has been reported.[5] The order of appearance of label from geranyl pyrophosphate and isopentenyl pyrophosphate was *trans*-farnesol, *trans*-farnesal, 2-*cis*-farnesal, 2-*cis*-farnesol, and a redox scheme for the isomerization was proposed. Whether or not aldehydes are free intermediates, our results and co-factor requirements would be in accord with a redox scheme.

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[4] Y. Machida and S. Nozoe, *Tetrahedron Letters*, 1972, 1969.

[5] L. Chayet, R. Pont-Lezica, C. George-Nascimento, and O. Cori, *Phytochemistry*, 1973, **12**, 95.