Biosynthesis of Tutin from (4*R*)-[4-$^3H_1$]Mevalonic Acid

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Summary Tutin from (4*R*)-[4-$^3H_1$]mevalonic acid retains one tritium atom at C-4, thus excluding a double 1–2 hydride shift during the cyclization steps of its biosynthesis.

INVESTIGATION of the biosynthesis of tutin (**1**) from labelled mevalonic acid[1,2] led to the proposal of an outline of the biogenesis of picrotoxane sesquiterpenoids; while the role of copaborneol as intermediate in this biosynthesis has been demonstrated,[3] no experimental results have been obtained so far on the mechanism of cyclization of *trans–cis*-farnesol pyrophosphate (**2**). The initial step of this cyclization can be regarded as interaction of the electrophilic centre at C-1 with the terminal or central double bond, leading to a germacrane or bisabolane cation, respectively.[4,5]

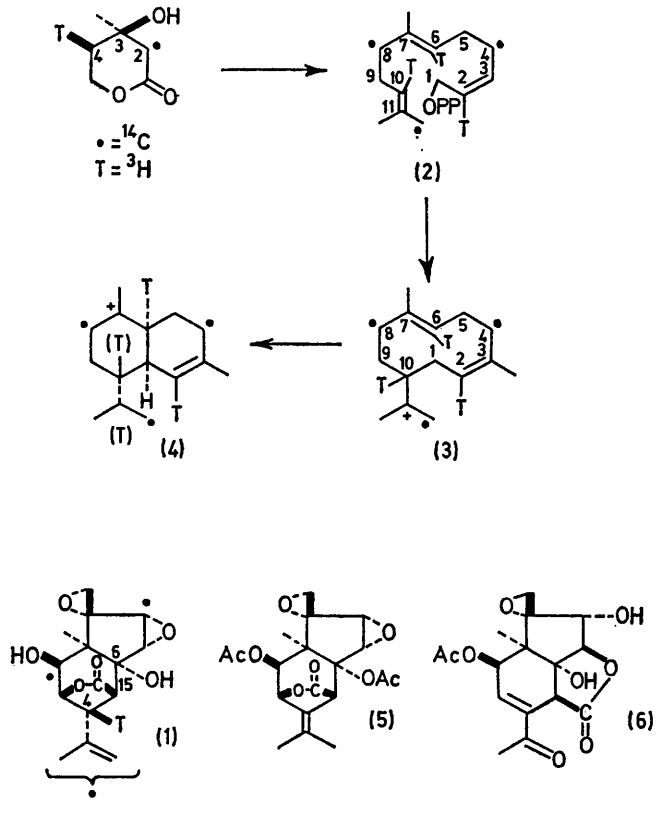
In the case of germacrane cation (**3**), in order to allow the formation of a bicyclic intermediate of the muurolane type, *e.g.* (**4**), through the interaction of the $\Delta^{6(7)}$ double bond with C-1, it is necessary to assume the transfer of the reactive centre from C-11 to C-1. It was suggested[4,6,7] that this can occur *via* (*a*) direct hydride shift from C-1 to C-11, (*b*) double 1–2 hydride shift from C-10 to C-11 and from C-1 to C-10, or (*c*) elimination of a proton from C-1 and intermediate formation of a cyclopropane ring; among these three alternatives, (*a*) and (*c*) imply the retention of only one tritium atom in tutin (**1**) biosynthetized from (4*R*)-[4-$^3H_1$] mevalonic acid; in fact tritium atoms at C-2 and C-6 of *trans–cis*-farnesol (**2**) are lost in the oxidative steps leading to the tertiary hydroxy-group at C-6 and to the C-15 carboxy-group in tutin. If a double hydride shift occurs (case *b*), the third tritium atom, too, is lost, a double bond being present at C-8 of tutin.

Potassium (3*RS*)-[2-^{14}C]mevalonate (0·1 mCi) and a mixture of potassium (3*R*,4*R*)-[4-$^3H_1$]- and (3*S*,4*S*)-[4-$^3H_1$]-mevalonate ($^3H/^{14}C$ 6·36) were fed to two-year-old plants of *Coriaria japonica* by the cotton-wick technique; after eight days, radioactive tutin (**1**) was isolated and purified to constant activity (0·015% incorp.; $^3H/^{14}C$ 2·20). The $^3H/^{14}C$ ratio of (**1**) clearly showed that two of the three tritium atoms of farnesol had been lost during the oxidative steps of the biosynthesis.

In order to locate the tritium label, tutin was transformed into neotutin diacetate (**5**),[2] showing the loss of 85% of the total tritium activity, the molar ^{14}C activity remaining constant.

Ozonolysis of 2-*O*-acetyltutin, followed by treatment of the product with alkali, gave the hydroxy-lactone (**6**)[2] for which again the loss of 85% of tritium activity was noticed.

These results show that most of the tritium label of the first isoprenic unit is bound to C-4 of tutin; besides confirming the proposed scheme[2] of biosynthesis of picrotoxane sesquiterpenoids, they demonstrate that the probable germacrane intermediate (**3**) does not rearrange through a



double 1—2 hydride shift. A similar conclusion has been reached by Arigoni and his co-workers for the biosynthesis of a microbial derivative of γ-cadinene.[8] The other possible cyclization mechanisms are under investigation.

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