# Mechanism of Alkylation during Sitosterol Biosynthesis in *Larix decidua*

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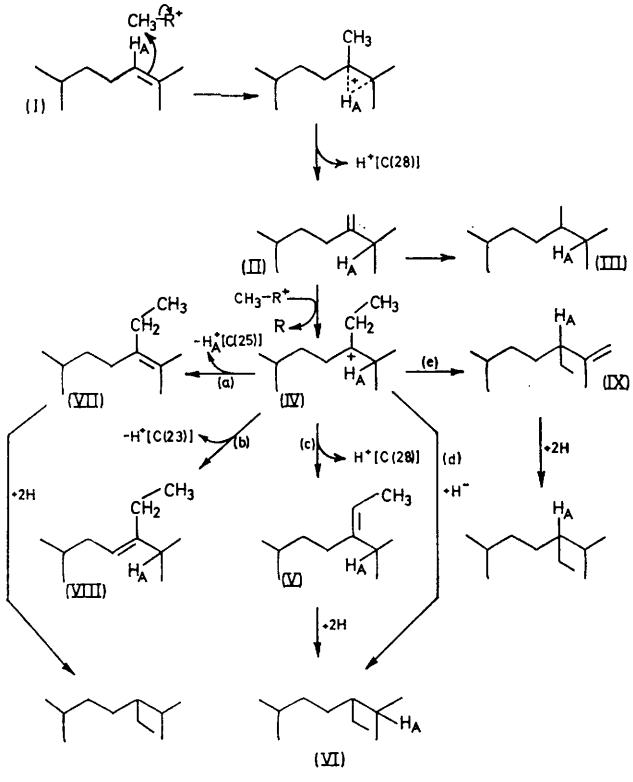
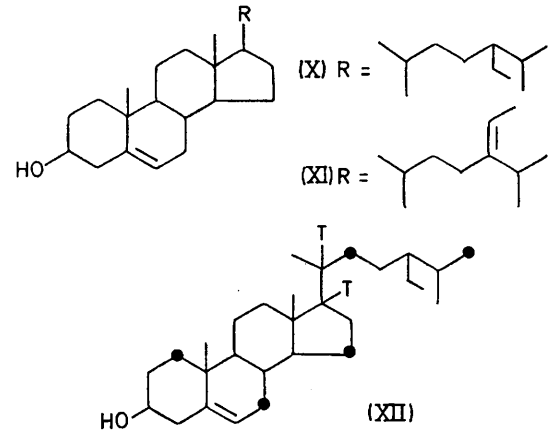
*Summary* Incorporation of [2-$^{14}$C-(4*R*)-4-$^{3}H_1$]mevalonic acid into sitosterol in *Larix decidua* demonstrates that during alkylation, the C-24 hydrogen of the $\Delta^{24}$ precursor is eliminated.

THE alkyl side chain at C-24 in phytosterols arises by transmethylation from *S*-adenosylmethionine.[1] A suggested[2] mechanism for this process involves hydrogen migration from C-24 to C-25 and formation of 24-methylene (II) and 24-ethylidene (V) compounds as precursors of C-24 methyl (III) and C-24 ethyl (VI) sterols, respectively (Scheme). In support of this Scheme, certain C-24 methyl and ethyl sterols biosynthesised by lower organisms in the presence of ($CD_3$)-methionine contain only two and four deuterium atoms, respectively.[3] In contrast to this, $C_{28}$ and $C_{29}$ alkyl-saturated sterols biosynthesised in other lower organisms retained three and five deuterium atoms, respectively[4,5] thus excluding the involvement of 24-methylene (II) and 24-ethylidene (V) intermediates in their biosynthesis. This has been explained[5] by invoking a $\Delta^{24(25)}$ intermediate, which is in agreement with the observation that the hydrogen at C-24 in the $\Delta^{24}$ sterol precursor is eliminated during stigmasterol biosynthesis in *Nicotina tabacum* and *Dioscorea tokoro*.[6]




SCHEME. $H_A$ *is derived from the* 4-pro-R *hydrogen of mevalonic acid.*

However, 24-methylene (II) and 24-ethylidene (V) sterols have not been conclusively shown to be intermediates in biosynthesis of any C-24 methyl- and ethyl- sterols in higher plants. Similarly, the hydrogen at C-24 in the precursor $\Delta^{24}$ sterol has only been conclusively shown to be retained at C-25 during ergosterol biosynthesis in yeast,[7] but never during biosynthesis of any C-24 saturated alkylsterol in higher plant tissue. In order to establish the fate of the C-24 hydrogen of the $\Delta^{24}$-sterol precursor in phytosterol biosynthesis in higher plants we report the incorporation of [2-$^{14}$C-(4*R*)-4-$^{3}H_1$]mevalonic acid into sitosterol (X) and 28-isofucosterol (XI) in *Larix decidua* leaves.




● = $^{14}$C; T = $^{3}$H

Young leaves of *Larix decidua* were chosen since sitosterol is the principal sterol[8] and 28-isofucosterol has also been radiochemically detected[9] in this tissue. We have now further characterized 28-isofucosterol as a minor component of *Larix decidua*, from a partly purified sterol fraction by g.l.c. and g.l.c.–m.s. (3% OV-17 column).

Chopped young leaves (2 g) of *Larix decidua* were incubated with [2-$^{14}$C-(4*R*)-4-$^{3}H_1$]mevalonate (10 $\mu$Ci$^{14}$C) overnight at 25° and the non-saponifiable lipids isolated. The sterols and squalene were obtained by t.l.c., and the squalene further purified after addition of carrier material, *via* the thiourea adduct (twice) and hexahydrochloride. Compounds (X) and (XI) were separated from the sterol fraction, after addition of carrier (XI) by t.l.c. on $AgNO_3$ impregnated silica gel. G.l.c. (3% OV 17 column) of the respective zones with trapping of samples at 1 min intervals for radioassay, indicated that all the radioactivity within each zone was associated with sitosterol and 28-isofucosterol, respectively. After dilution with carrier material, sitosterol and 28-isofucosterol were recrystallised to constant specific radioactivity. Since the $^{3}$H : $^{14}$C atomic ratio (Table) of the recrystallised sitosterol (X) was between 2 : 5 and 3 : 5, portions of (X) were further processed by (i) formation of the dibromide derivative and regeneration of the free sterol in ether with zinc–acetic acid and recrystallization[10] and (ii) treatment with Jones reagent yielding 24*R*-ethylcholest-4-en-3,6-

dione.[11] The latter was further transformed after dilution with carrier material by treatment with zinc and acetic acid into 24*R*-ethylcholestane-3,6-dione,[12] m.p. 186—188°; *m/e* 428.

The $^3H:^{14}C$ ratios for the sitosterol purified *via* dibromide, the 24*R*-ethyl-cholest-4-ene-3,6-dione and the 24*R*-ethylcholestane-3,6-dione are in good agreement (2:5), and indicate the presence of only two tritium atoms in the biosynthesised sitosterol. By analogy with cholesterol biosynthesis in animals,[13] the compound (X) should be labelled as shown (XII). The higher $^3H:^{14}C$ ratio observed for the recrystallized sitosterol which had not been purified further indicates that a radioactive impurity, with a higher $^3H:^{14}C$ ratio (possibly the corresponding $\Delta^7$ sterol), had been carried through the recrystallization.

The present results for sitosterol (X) are in agreement with those of Tomita and his co-workers[6] for stigmasterol formation in *Nicotiana* and *Dioscorea* tissue cultures, and support the operation of route (a) (Scheme) involving a $\Delta^{24(25)}$ intermediate (VII) but not routes (b), (c), (d), or (e), during sitosterol biosynthesis. A $^3H:^{14}C$ ratio of 3:5 for 28-isofucosterol (XI) ≡ (V) is in agreement with the established[14] operation of route (c) in its biosynthesis. The present results indicate that 28-isofucosterol is not on the major pathway to (X) in *Larix decidua*, at least not without isomerization to a $\Delta^{24}$ structure. In view of this, other earlier reports[15] of the incorporation of [2-$^{14}$C-(4*R*)-4-$^3H_1$]mevalonic acid into higher plant sterols, which gave inconclusive results will require further careful reinvestigation.

TABLE. *Incorporation of* [2-$^{14}$C-(4*R*)-4-$^3H_1$]*mevalonate into sitosterol and* 28-*isofucosterol in* Larix decidua

| Compound | Specific radioactivity (in d.p.m. $^{14}C$ mg$^{-1}$) | $^3H:^{14}C$ radioactivity ratio[a] | $^3H:^{14}C$ atomic ratio (based on squalene) |
|---|---|---|---|
| Squalene | | 7·54 | |
| Sitosterol (X) (recrystallized) | 228 | 3·67 | 2·43:5 |
| Sitosterol (X) (purified *via* dibromide) | 350 | 3·36 | 2·23:5 |
| 24*R*-Ethylcholest-4-ene-3,6-dione | 340 | 3·33 | 2·21:5 |
| 24*R*-Ethylcholestane-3,6-dione[b] | 174 | 3·13 | 2·08:5 |
| 28-Isofucosterol (XI) (recrystallized) | 253 | 4·53 | 3·00:5 |

[a] The specific radioactivities and radioactivity ratios quoted are the mean of three sequential crystallisations in each case. [b] Diluted with carrier material before recrystallization.

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