# Mechanism of Biosynthesis of the Ethyl Side-chain at C-24 of Stigmasterol in Tissue Cultures of *Nicotiana tabacum* and *Dioscorea tokoro*

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*Summary* On the basis of hydrogen elimination at C-24 during biosynthesis of stigmasterol in tissue cultures of *N. tabacum* and *D. tokoro*, the $\Delta^{24(25)}$ compound as an intermediate is involved in stigmasterol biosynthesis.

IT has been established that the ethyl and ethylidene side-chains at C-24 of sterols, characteristic of phytosterols, are derived by double transmethylation from adenosylmethionine.[1-3] The first transfer of a methyl group to a $\Delta^{24(25)}$ double bond leads to C-24 methylene compound (II) with migration of a hydrogen atom from C-24 to C-25[4,5] and the second transmethylation to the $\Delta^{24(28)}$ double bond of the intermediate (II) would form carbonium ion (III), which could conceivably be stabilized by several routes (a, b, c and d).[6] It was thought that the ethylidene compound (IV) is a precursor for C-24 ethyl-sterols, and this has been proved in the case of poriferasterol synthesized by *Ochromonas malhamensis*.[7] On the other hand, Lenfant *et al.* showed that $\Delta^{22}$-stigmasten-3$\beta$-ol in *Dictyostelium discoiseum* is synthesized by a mechanism which does not involve a C-24 ethylidene intermediate.[8]

In previous communications[9,10] we have reported that the C-24 ethylidene derivative is excluded from the biosynthesis of chondrillasterol, $\Delta^{7}$-chondrillastenol, and poriferasterol in Chlorella. Moreover, we have suggested that a $\Delta^{24(25)}$ intermediate (V) might be formed for stabilization of the carbonium ion (III). As shown in the Scheme, when stigmasterol (I) is synthesized by route a, b, or d, the hydrogen atom at C-24 derived from the 4-(*pro-R*)-hydrogen of mevalonic acid (MVA) should be retained at C-25, whereas if stigmasterol is synthesized by route c the hydrogen atom should be eliminated during biosynthesis. We now report the mechanism of biosynthesis of the ethyl side-chain on the basis of hydrogen elimination at C-24 during biosynthesis of stigmasterol from cycloartenol.

Cells of *N. tabacum* tissue cultures, grown for a week in Linsmeier–Skoog medium containing 3*R*-[2-$^{14}$C, (4*R*)-4-$^{3}H_1$]-MVA ($^{14}C = 10\ \mu Ci$, $^{3}H/^{14}C = 9{\cdot}1$) were extracted with methanol. A mixture of phytosterols was isolated from unsaponifiable lipid by preparative t.l.c. on silica gel and was acetylated. Stigmasteryl acetate was obtained from the mixture by preparative t.l.c. on silver nitrate–silica gel and was finally recrystallized to constant specific radioactivity after addition of carrier stigmasteryl acetate.

It has been demonstrated that a $C_{27}$ sterol such as cholesterol, derived from 3*R*-[2-$^{14}$C, (4*R*)-4 $^{3}H_1$]-MVA, is labelled with three tritium atoms at C-17, C-20, and C-24[11,12] but the stigmasteryl acetate obtained here was labelled with two tritium atoms and five $^{14}$C atoms. The ozonide of the stigmasteryl acetate was decomposed with zinc powder in acetic acid and the side-chain fragment (VII), isolated by steam distillation from the reaction mixture, was converted into the dimedone derivative, m.p. 127—129°, which was recrystallized to constant specific activity. As

shown in the Table, 95% of the tritium present at C-24 was lost during transmethylation. Cycloartenol and 24-methylenecycloartanol, precursors for phytosterols,[13,14] were

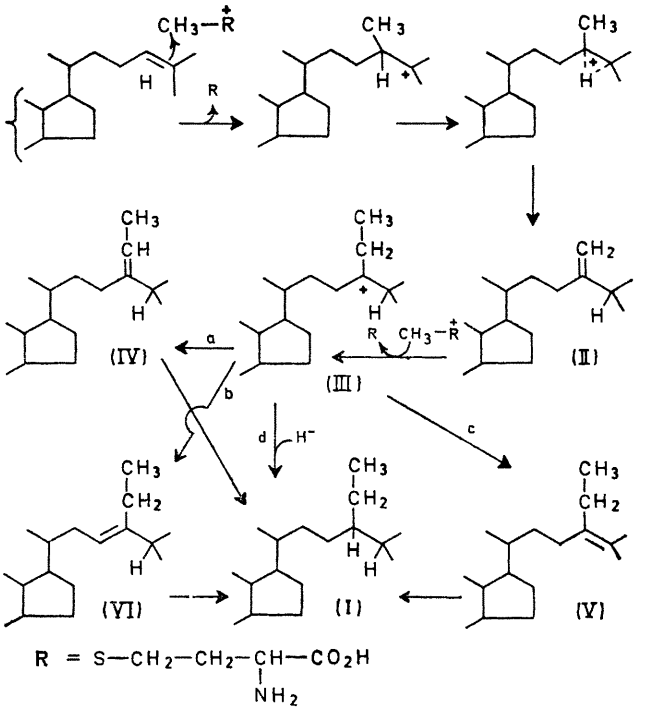
*The $^3H/^{14}C$ ratios for cycloartenol, 24-methylenecycloartenol, and stigmasterol derived from [2-$^{14}$C, (4R)-4 $^3$H]-MVA and their degradation products*

| | $^3H:^{14}C$ (d.p.m.) | $^3H:^{14}C$ (atomic) Exp. | Theor. |
|---|---|---|---|
| Cycloartenyl acetate[a] .. .. | 9·15 | 6·03 : 6 | 6 : 6 |
| 24-Methylenecycloartanyl acetate[a] .. .. .. | 9·08 | 5·98 : 6 | 6 : 6 |
| 24-Oxocycloartanol[a] .. .. | 7·52 | 4·95 : 6 | 5 : 6 |
| Stigmasteryl acetate[a] .. .. | 3·90 | 2·14 : 5 | 2 : 5 |
| Stigmasteryl acetate[b] .. .. | 5·40 | 2·17 : 5 | 2 : 5 |
| Dimedone deriv. of (VII)[a] .. | 0·45 | 0·049 : 1 | 0 : 1 |

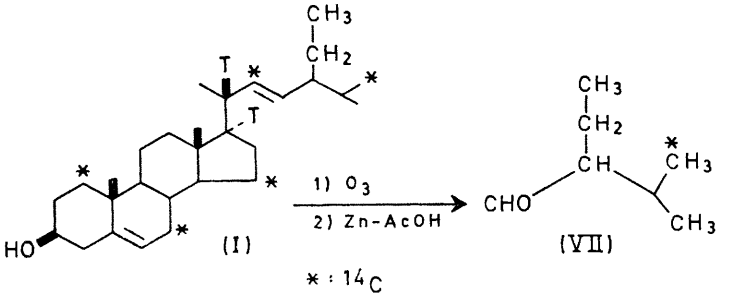
[a] Derived from MVA, $^3H/^{14}C$ = 9·1 in *N. tabacum*.
[b] Derived from MVA, $^3H/^{14}C$ = 12·4 in *D. tokoro*.

isolated, respectively, as their acetates in the manner described by Benveniste, Hirth, and Ourisson.[15] These acetates were labelled with six $^3$H and six $^{14}$C atoms, and 24-oxocycloartanyl acetate, prepared by ozone degradation of 24-methylenecycloartanyl acetate, lost one tritium atom on alkali treatment. Thus, a tritium atom at C-24 of cycloartenol was not lost in 24-methylenecycloartanol and migration of a tritium atom from C-24 to C-25 takes place in the first transmethylation. Therefore, elimination of the tritium must occur in the second transmethylation. The results show that stigmasterol is synthesized by the route c.

Moreover, stigmasteryl acetate, isolated from tissue cultures of *D. tokoro* grown with 3*R*-[2-$^{14}$C, (4*R*)-4-$^3H_1$]-MVA ($^{14}$C = 10 $\mu$Ci, $^3H/^{14}C$ = 12·4), was labelled with two $^3$H and five $^{14}$C atoms. Tissue cultures of *D. tokoro* were incubated with [24-$^3H_1$]cycloartenol (VIII) (10 $\mu$Ci) for three weeks, and sapogenins and stigmasterol were isolated as described previously.[16] The sapogenins obtained had radioactivity[17] but stigmasterol was not radioactive.




SCHEME




These results are consistent with those observed in *N. tabacum* tissue cultures.

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