Metabolism of C_{28} Phytosterols in the Insect *Tenebrio Molitor*: Migration of the C-25 Hydrogen Atom to C-24

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During the conversion of 24-methylenecholesterol into cholesterol by *Tenebrio molitor* larvae the C-25 hydrogen migrates to the C-24 position.

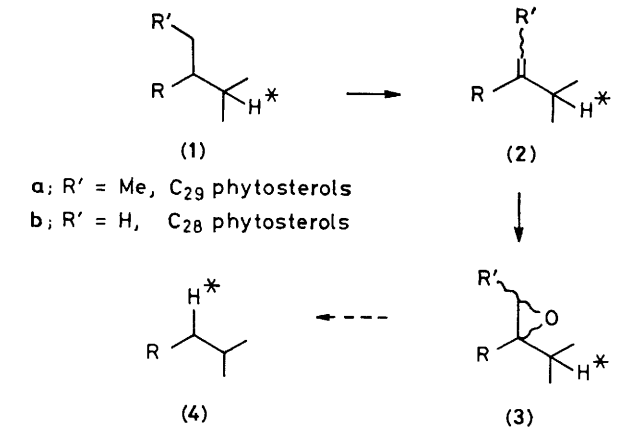
Cholesterol can be obtained by many phytophagous insects through the dealkylation of C_{28} and C_{29} phytosterols that they find in their diet.[1] The mechanism of this process has been thoroughly investigated for C_{29} phytosterols for which the intermediacy of the stereoisomeric 24,28-ethylidene compounds (**2a**) (Scheme 1)[2] and 24,28-epoxides (**3a**)[3] has been demonstrated. A peculiar feature in the above metabolic sequence is the hydrogen migration from the C-25 to the C-24 position during the conversion of the 24,28-epoxides (**3a**) into cholesterol (**4**)[4] as illustrated in Scheme 1.

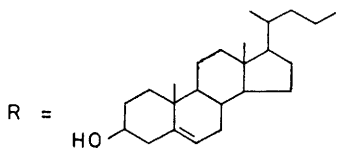
We have recently observed[5] that the dealkylation of C_{28} phytosterols seems to proceed in a similar way: in fact both 24-methylenecholesterol (**2b**) and the corresponding 24,28-epoxides (**3b**) are converted into cholesterol (**4**) by *Tenebrio molitor* larvae. Moreover, 24-methylenecholesterol epoxide has been shown to be an intermediate in the formation of cholesterol in *Schistocerca* mid-gut microsomes.[6]

We now report our results on the mechanism of this process, *i.e.* the demonstration that the hydrogen shift from the C-25 to the C-24 position occurs also in the C_{28} phytosterol dealkylation.

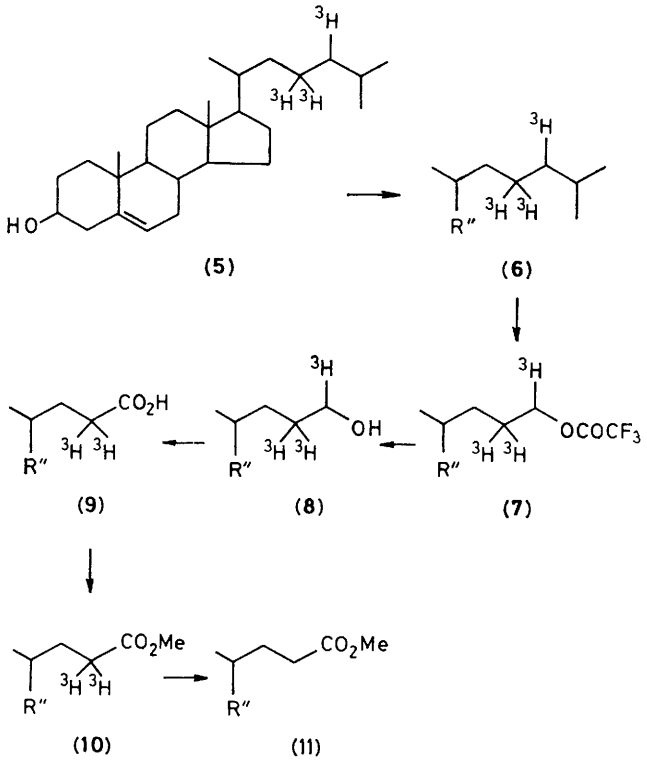
A mixture of [23,23,25-$^3H_3$]-24-methylenecholesterol (5.96 $\times$ 10^7 dpm of ^3H, spec. act. 2.64 $\times$ 10^7 dpm/mg), synthesized from [23,23,25-$^3H_3$]-24-oxocholesterol,[7] and [4-^{14}C]sitosterol (7.86 $\times$ 10^6 dpm of ^{14}C, spec. act. 2.85 $\times$ 10^8 dpm/mg, the Radiochemical Centre, Amersham) as internal standard, was fed to 500 young *Tenebrio molitor* larvae. After four days the larvae were sacrificed and the unsaponifiable fraction, obtained as previously described,[3] was benzoylated. Cholesteryl benzoate was obtained pure by sequential argentation t.l.c. (to remove the unconverted 24-methylenecholesteryl benzoate) and preparative h.p.l.c. (Waters μBondapack-C_{18} column, flow rate 2.5 ml/min, solvent MeOH–H_2O 97:3), which removed the residual sitosteryl benzoate.

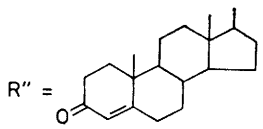
The labelled cholesteryl benzoate, diluted with cold material, was subjected to alkaline hydrolysis and to Oppenauer oxidation to yield labelled cholest-4-en-3-one (**6**) (Scheme 2). Trifluoroperacetic acid oxidation of (**6**)[8] gave 24-trifluoroacetoxychol-4-en-3-one (**7**) with loss of the terminal isopropyl group; hydrolysis to the 24-hydroxy-compound (**8**) followed by oxidation with pyridinium dichromate (PDC)[9] afforded the 24-carboxy-derivative (**9**); esterification with diazomethane and exchange of the obtained methyl 3-oxochol-4-en-24-oate (**10**) with MeOH/MeO^- gave the methyl ester





Scheme 1





Scheme 2

Table 1. Specific activities and $^3H/^{14}C$ ratios of cholesterol and its chemical degradation products.

Compounds	dpm of ^{14}C/mmol	$^3H/^{14}C$[c]
(5)	1.13×10^5	13.6
(6)	1.14×10^5	13.7
(8)	1.15×10^5	13.2
(10)[a]	1.08×10^5	8.2
(11)[b]	1.10×10^5	0.9

[a] Methyl 3-oxochol-4-en-24-oate before MeOH/MeO^- exchange.
[b] Methyl 3-oxochol-4-en-24-oate after MeOH/MeO^- exchange.
[c] Ratio of dpm of ^3H/mmol to dpm of ^{14}C/mmol.

(**11**) with almost complete loss of tritium.

Each product of the above sequence was crystallized to constant specific activity and $^3H/^{14}C$ ratio and the values obtained are reported in Table 1.

The total retention of the tritium label during the peracetic acid oxidation of (**6**) shows the absence of label at C-25; moreover the loss of 40% of tritium during the PDC oxidation of (**8**) is indicative of the presence of tritium at C-24. The virtually complete loss of the remaining tritium by MeOH/MeO^- exchange of (**10**) indicates that this residual tritium is present at C-23.

These results clearly demonstrate that during the metabolism of 24-methylenecholesterol by *Tenebrio molitor*, the C-25 hydrogen migrates to the C-24 position and they also provide further evidence for the strict similarity of the de-ethylation process of C_{29} phytosterols and the demethylation process of their C_{28} analogues.†

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† A recent communication (S. Maruyama, Y. Fujimoto, M. Morisaki, and N. Ikekawa, *Tetrahedron Lett.*, 1982, **23**, 1701) actually confirms 24-methylenecholesterol as an intermediate and suggests a possible migration of the hydrogen from the C-25 to the C-24 position during the demethylation of campesterol (**1b**) in the insect *Bombyx mori*.