The Fate of the C-25 Hydrogen of 28-Isofucosterol during Conversion into Cholesterol in the Insect *Tenebrio molitor*

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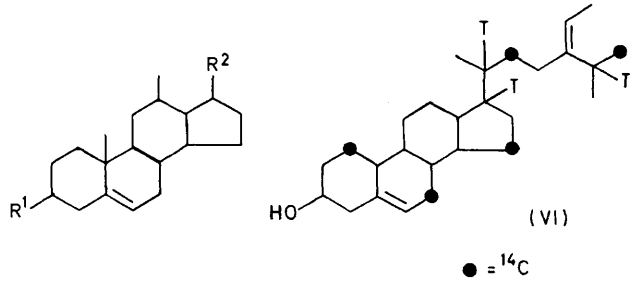
Summary The C-25 hydrogen of 28-isofucosterol is retained, probably at C-24, during dealkylation to cholesterol in the insect, *Tenebrio molitor*.

MANY species of phytophagous and omnivorous insects can dealkylate C_{28} and C_{29} sterols yielding cholesterol (IVa).[1] Fucosterol (II) and desmosterol (III) have been implicated

as intermediates in sitosterol (I) dealkylation in several insect species.[2,3,4] Although the exact dealkylation mechanism is obscure, a reaction sequence for the transformation has been suggested[3] (I → II → III → IVa). The above findings support the suggestion[2] that the process of C-24 dealkylation of sterols in insects may in part be the reverse of sterol C-24 alkylation in plants.[5] It has been established that the transformation in plants of a Δ^{24}-sterol into a 24-ethylidene compound involves hydrogen migration from C-24 to C-25 (see preceding communication).[6] The fate of the C-25 hydrogen in the 24-ethylidene compound, 28-isofucosterol (Va) during dealkylation to (IVa) in the insect, *Tenebrio molitor* (yellow mealworm) is now reported.



(VI)

● = ^{14}C

	R^1	R^2
(I)	OH	
(II)	OH	
(III)	OH	
(IVa)	OH	
(IVb)	Ac	
(Va)	OH	
(Vb)	Ac	

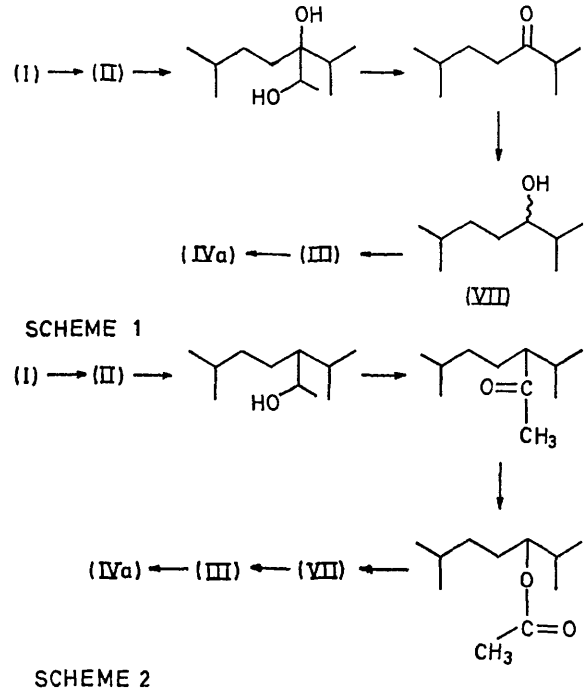
28-Isofucosterol (VI) ($7{\cdot}0 \times 10^4$ d.p.m. ^{14}C) biosynthetically prepared by incorporation of [2-^{14}C-(4*R*)-4-$^{3}H_1$]-mevalonate into larch leaves (preceding communication)[6] was deposited onto a small amount of finely ground oatmeal and fed to *ca.* 300 young larvae of *Tenebrio molitor*, which had been starved for 36 h. When the food had been consumed (1½—2 days), the insects were macerated in ethanol and

TABLE

Compound	$^{3}H:^{14}C$ radioactivity ratio[a]	$^{3}H:^{14}C$ atomic ratio (based on squalene)
Squalene (from *Larix decidua*) ..	7·54	6:6
Administered 28-Isofucosterol (from *Larix decidua*) (Va) ..	4·53	3·00:5
Cholesteryl acetate (isolated from insects) (IVb)	4·51	2·99:5

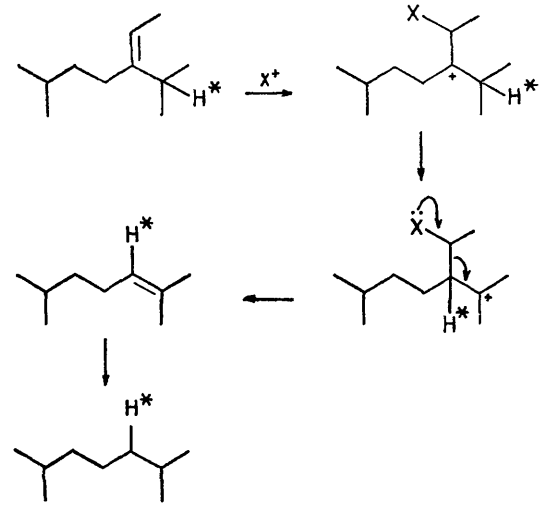
[a] The radioactivity ratios quoted are the mean of three sequential crystallisations in each case.

subjected to alkaline saponification. The sterol fraction obtained after chromatography of the non-saponifiable material was diluted with carrier (Va) (3 mg), acetylated, subjected to t.l.c. on $AgNO_3$ impregnated silica gel and the acetates of (Vb) ($4{\cdot}95 \times 10^3$ d.p.m. ^{14}C) and (IVb) ($1{\cdot}41 \times 10^4$ d.p.m. ^{14}C) eluted. Preparative g.l.c. (3% SE-30) of a portion of the cholesteryl acetate fraction, with collection of fractions at 1 min intervals for radioassay, showed that (IVb) was the only labelled component and that no reduction of (Va) to sitosterol (I) occurs in the insect. Therefore, the remaining cholesteryl acetate was diluted with carrier material and recrystallised to constant specific radioactivity (246 d.p.m. $^{14}C\ mg^{-1}$).



SCHEME 1

SCHEME 2



X = R—O$^+$; R—S$^+$; R_2—N$^+$

SCHEME 3

The $^{3}H:^{14}C$ atomic ratio for the cholesterol isolated from the insect approaches 3:5 and shows that all the tritium atoms of the administered (Va) are retained in (IVa) after dealkylation (Table). This result indicates that the C-25 hydrogen atom must migrate during dealkylation, since

(III) has been implicated[2,4] as an intermediate in this process.

By analogy with cholesterol side-chain cleavage in vertebrates[7] and the suggested[8] degradation of progesterone in certain microbial systems, we had earlier considered Schemes 1 and 2 as possible mechanisms for dealkylation of (I). To test the possible intermediacy of (VII), common to either scheme, [24-*R* and 24-*S*- 7-$^3H_2$]-(VII)[9,10] were separately fed to *T. molitor*, but the negligible conversion of both into cholesterol could be due to the poor uptake of (VII) from the insect gut or suggests an alternative mechanism for dealkylation.

A mechanism for dealkylation consistent with the above results is given in Scheme 3; this involves migration of the C-25 hydrogen to C-24. The conversion of fucosterol-24, 28-epoxide into (III) by BF_3 etherate has been interpreted[11] in terms of a similar mechanism.

Further studies are in progress to locate the C-25 hydrogen of sitosterol in the cholesterol resulting from dealkylation.

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