Migration of the C-25 Hydrogen of Clionasterol to the C-24 Position during Dealkylation by the Insect, Tenebrio molitor

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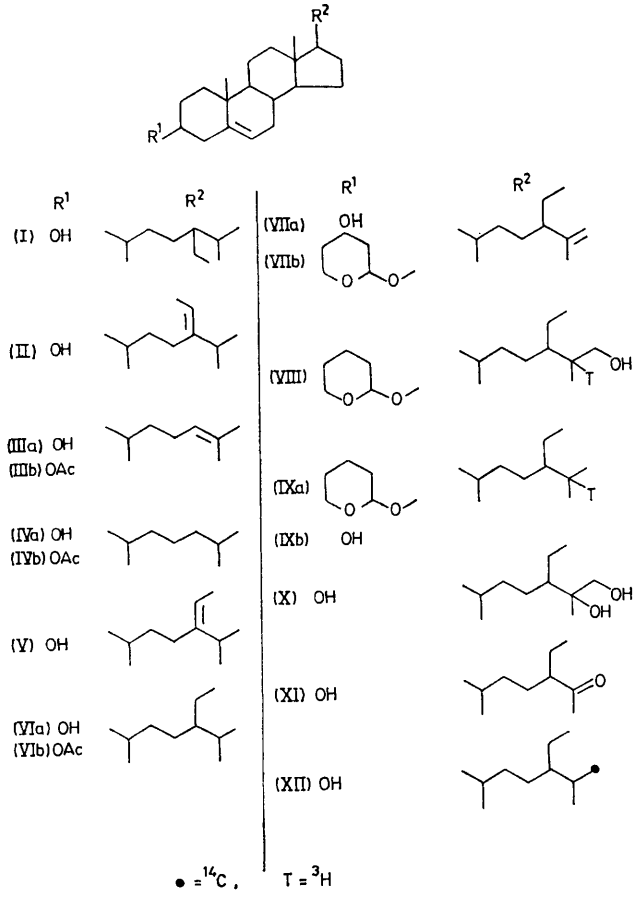
Summary Dealkylation of synthetic [25-^{3}H,26-^{14}C]clionasterol to desmosterol by the insect, *Tenebrio molitor*, involves migration of the C-25 hydrogen to the C-24 position.

MANY phytophagous and omnivorous insect species dealkylate dietary C_{28} and C_{29} plant sterols yielding cholesterol (IVa). Good evidence in support of the reaction sequence (I) → (II) → (IIIa) → (IVa) for sitosterol (I) dealkylation has been furnished.[1] We have previously reported that the C-25 hydrogen of 28-isofucosterol (V) is retained during transformation into cholesterol (IVa) by the insect, *Tenebrio molitor*.[2] Since desmosterol (IIIa) has been implicated as an intermediate in dealkylation, the C-25 hydrogen must have undergone migration, possibly to C-24 in cholesterol (IVa).

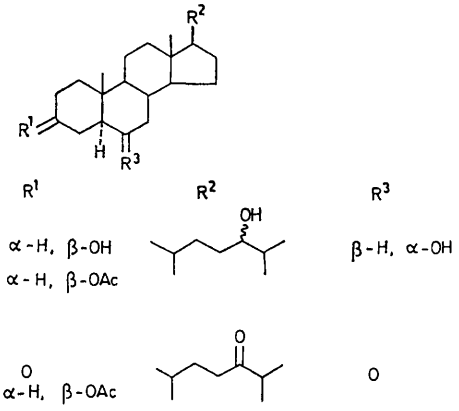
Conclusive evidence for the migration of the C-25 hydrogen of clionasterol (VIa) to C-24 during dealkylation into cholesterol (IVa) is now reported. Clionasterol was chosen as substrate because a suitable starting material for the synthesis, *viz.* clerosterol[3] (VIIa), is readily available. Since the side chain of desmosterol (IIIa) is more amenable to chemical reaction than that of cholesterol (IVa), the doubly labelled sterol was administered to the insects together with the vertebrate hypocholesterolemic agent, triparanol, which causes accumulation of (IIIa)[4]

Hydroboration[5] of clerosteryl tetrahydropyranyl ether (VIIb) with [^{3}H]diborane gave (VIII), and $LiAlH_4$ reduction of the derived C-26 tosylate furnished (IXa), from which [25-^{3}H]clionasterol (IXb) was obtained by acid treatment. Base equilibration of the derived aldehyde from a portion of (VIII) showed that at least 98% of the tritium label was present at C-25. [26-^{14}C]Clionasterol was also prepared from clerosterol (VIIa) by hydroxylation of the Δ^{25} bond with OsO_4 yielding the 25,26-diol (X), from which (XI) was produced by periodate cleavage. Wittig condensation of (XI) with [^{14}C]methyltriphenylphosphonium iodide in the presence of Bu^nLi gave [26-^{14}C]clerosterol, which was hydrogenated[6] using Wilkinson's catalyst yielding [26-^{14}C]clionasterol (XII).



Young larvae of *Tenebrio molitor* (*ca.* 1500) were fed for 10 days on a diet containing 0·26% triparanol, and were maintained for a further 10 days on a diet coated with [25-^{3}H,26-^{14}C]clionasterol and 0·26% triparanol. The insects were macerated in ethanol and subjected to alkaline saponification. The sterol fraction isolated after chromatography was acetylated, and separated into bands corresponding to the acetates of cholesterol (IVb) plus clionasterol (VIb), and desmosterol by t.l.c. on $AgNO_3$–silica gel.

An aliquot portion of the substrate [25-^{3}H, 26-^{14}C]-clionasterol was diluted with carrier material and recrystallised to constant specific radioactivity. The acetates of cholesterol (IVb) and clionasterol (VIb) were separated by preparative g.l.c. and the former diluted with carrier material prior to recrystallisation. A portion of the desmosteryl acetate (IIIb) after addition of carrier material was recrystallised to constant specific radioactivity, while the remainder was also diluted with carrier and hydroborated yielding a mixture of diol (XIIIb) and triol (XIIIa), which were recrystallised. After further addition of carrier material to both (XIIIb) and (XIIIa), the compounds were oxidised to the corresponding dione (XIVb) and the trione (XIVa) with CrO_3–pyridine[7] and Jones reagent, respectively, followed by recrystallisation.



The ^{3}H:^{14}C atomic ratios (Table) of the acetates of cholesterol (IVb) and desmosterol (IIIb) are in close agreement with that for the administered clionasterol (VIa) and indicate that the C-25 tritium is retained during dealkylation. The slight differences between these ratios is probably due to kinetic isotope effects, as indicated by the higher ratio for the recovered clionasterol acetate (VIb). The chemical transformation of the desmosteryl acetate (IIIb) into the diol (XIIIb) and triol (XIIIa) occurs with almost complete retention of tritium, which is essentially eliminated upon oxidation to the corresponding dione (XIVb) and trione (XIVa), respectively. These results demonstrate that the tritium atom in desmosterol is present at C-24, so that tritium migration from C-25 to C-24 has occurred during dealkylation of the clionasterol (IXb).

TABLE

Transformation of [25-^{3}H, 26-^{14}C]+clionasterol into desmosterol and cholesterol by *Tenebrio molitor*

	Specific radioactivity (d.p.m. ^{14}C mg^{-1})	^{3}H:^{14}C Radioactivity ratio	^{3}H:^{14}C Atomic ratio (based on the administered clionasterol)
Administered (VIa) ..	492	14·11	1:1
(VIb) recovered from insects (ex. g.l.c.) ..	—	14·82	1·05:1
(IVb) isolated from insects	478	14·93	1·05:1
(IIIb) isolated from insects	2173	13·62	0·96:1
(XIIIb)	4268	13·07	0·92:1
(XIIIa)	4406	12·25	0·86:1
(XIVb)[a]	—	0·66	0·04:1
(XIVa)[a]	3450	0·36	0·02:1

[a] (XIIIa) and (XIIIb) were diluted with carrier material before oxidation.

Fucosterol-24,28-epoxide has been implicated as a probable intermediate in the conversion of fucosterol into

cholesterol (IVa) in insects.[8] This can also be rationalised by invoking a mechanism involving hydride migration from C-25 to C-24, analogous to that previously proposed[9] for the chemical transformation of fucosterol-24,28-epoxide into desmosterol by BF_3, Et_2O.

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