Conversion of Isofucosterol-(24*R*,28*S*)-epoxide into Cholesterol in the Insect *Tenebrio molitor*

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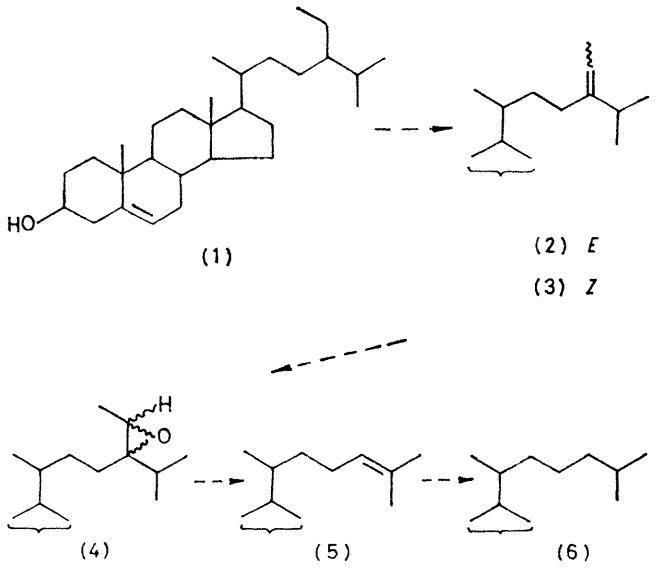
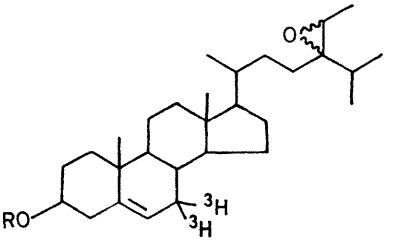
Summary *Tenebrio molitor* larvae convert [7-$^3H_2$]-(24*R*,28*S*)-24,28-epoxystigmast-5-en-3β-ol, but not its (24*S*,28*R*)-diastereoisomer, into cholesterol.

PHYTOPHAGOUS insects have developed a metabolic pathway to obtain cholesterol (**6**) from phytosterols, such as sitosterol (**1**), which they find in their diet.[1] The generally accepted scheme for this process[2,3] involves a 24(28)-double bond intermediate (**2**), which is transformed into cholesterol *via* epoxidation to (**4**), followed by elimination of a C-2 unit to form desmosterol (**5**) and saturation of the 24(25)-double bond of this last compound (Scheme). In *Tenebrio molitor* this dealkylation process occurs with the formation of isofucosterol (**3**)[4] as well as fucosterol. We now report the absolute stereochemistry of the epoxide involved in the transformation of isofucosterol into cholesterol in *Tenebrio molitor*.



SCHEME

Epoxidation[5] of [7-$^3H_2$]isofucosteryl acetate (synthesized according to the published procedure)[6] with *m*-chloroperbenzoic acid yielded a mixture of [7-$^3H_2$]-(24*R*,28*S*)- and [7-$^3H_2$]-(24*S*,28*R*)-3β-acetoxy-24,28-epoxystigmast-5-enes (**7a**) and (**8a**), which were separated by preparative t.l.c. (benzene–ethyl acetate 99:1, five elutions) and hydrolysed to the corresponding free sterols (**7b**) and (**8b**).[5] The



(**7**)**a**; (24*R*,28*S*), R = Ac (**8**)**a**; (24*S*,28*R*), R = Ac
b; (24*R*,28*S*), R = H **b**; (24*S*,28*R*), R = H.

absolute configuration of (**7a**) and (**8a**) was determined from the corresponding 24-methoxy-28-alcohols obtained from each epoxide by acid catalysed methanolysis, using a g.l.c. modification[6,7] of Horeau's method.

A mixture of (**7b**) ($1{\cdot}23 \times 10^8$ d.p.m., specific activity 24·8 mCi/mmol) and [4-^{14}C]sitosterol (Radiochemical Centre

TABLE Total radioactivity and $^{3}H/^{13}C$ ratios of the administered precursors and of the isolated cholesterol

Expt	Administered [7-$^{3}H_{2}$]epoxides d p m of ^{3}H	Administered [4-^{14}C]sitosterol d p m of ^{14}C	$^{3}H/^{14}C$	Cholesteryl acetate d p m of ^{3}H	Cholesteryl acetate d p m of ^{14}C	$^{3}H/^{14}C$
1	(**7b**) $1{\cdot}23\times10^{8}$	$6\,36\times10^{6}$	19 3	$7\,70\times10^{6}$	$2\,18\times10^{5}$	35 3
2	(**8b**) $1\,33\times10^{8}$	$9\,14\times10^{6}$	14 6	$7{\cdot}90\times10^{5}$	$3\,19\times10^{5}$	2 5

Amersham, $6\,36\times10^{6}$ d p m, sp act 53 6 mCi/mmol), deposited onto 320 mg of finely ground oatmeal, was fed to 225 young larvae (3 2 g) of *Tenebrio molitor*, which had been starved for 48 h (expt 1, see Table) After four days the larvae were sacrificed, macerated in ethanol, and submitted to mild alkaline hydrolysis (0·25% methanolic KOH) The unsaponifiable fraction was acetylated and separated by preparative t l c on silica gel (developed with benzene–ethyl acetate 98:2) into two bands The less polar band corresponded to the acetates of sitosterol plus cholesterol, whereas the more polar band corresponded to the acetate of the unconverted epoxide The acetates of sitosterol and cholesterol were separated by preparative g l c (2 5% SE-30, T_{C} 220 °C) Cholesteryl acetate was diluted with cold material and repeatedly crystallized to constant specific activity In expt 2, (**8b**) ($1{\cdot}33\times10^{8}$ d p m, sp act 24·8 mCi/mmol) and [4-^{14}C](**1**) ($9\,14\times10^{6}$ d p m) deposited onto 300 mg of oatmeal were administered to 225 larvae (3 0 g) Cholesteryl acetate was isolated according to the procedure of expt 1 and crystallized to constant specific activity (see Table)

Comparison of the cholesteryl acetate $^{3}H/^{14}C$ ratios in the two experiments clearly shows that the (24*R*,28*S*)-epoxide (**7b**) is converted into (**6**) in *ca* ten times higher yield than its (24*S*,28*R*)-isomer (**8b**), suggesting that (**7b**) is the actual precursor of cholesterol

It is interesting to note that in *Bombyx mori* fucosterol is epoxidized from the 24*re*,28*si* face,[3] whereas in *Tenebrio molitor* epoxidation of isofucosterol occurs from the 24*si*,-28*si* face, this indicates a somewhat different steric approach of furosterol and isofucosterol molecules to the epoxidizing enzymatic system †

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† *Added in proof* Ikekawa and his colleagues have recently published a paper (Y Fujimoto, K Murakami, and N Ikekawa, *J. Org Chem*, 1980, **45**, 566) in which the configuration previously assigned (ref 3) to the 24,28-fucosterol epoxides is reversed

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