Fucosterol-24,28-epoxide, as a Probable Intermediate in the Conversion of β-Sitosterol to Cholesterol in the Silkworm

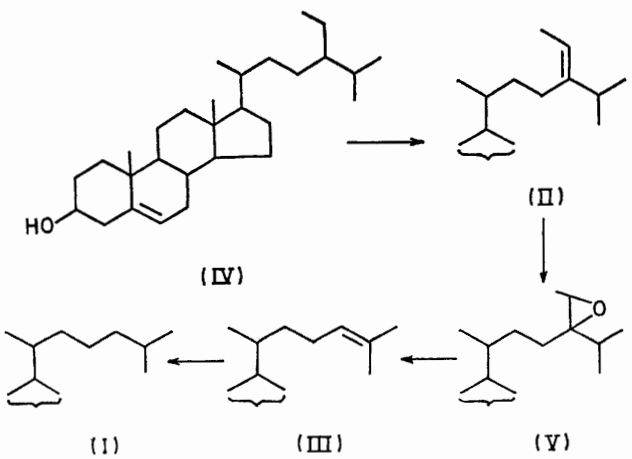
By MASUO MORISAKI, HIROSHI OHTAKA, MARI OKUBAYASHI, and NOBUO IKEKAWA*
(*Laboratory of Chemistry for Natural Products, Tokyo Institute of Technology, Ohokayama, Meguro-ku, Tokyo, Japan*)
and YASUHIRO HORIE and SHOICHI NAKASONE
(*Sericultural Experiment Station, Suginami-ku, Tokyo, Japan*)

Summary ^{3}H-Fucosterol-24,28-epoxide (V) was effectively incorporated into cholesterol (I) in the silkworm; it was also trapped in the insect as a probable intermediate in conversion of fucosterol (II) into cholesterol (I).

DEALKYLATION of the phytosterol side chain to give cholesterol (I) is of vital importance in the phytophagous insect.[1] Although fucosterol (II) and desmosterol (III) have been identified as intermediates between β-sitosterol (IV) and (I),[2] the precise mode of the conversion is unknown. Recently, we have found that fucosterol-24,28-epoxide (V) is transformed into (III) by treatment with BF_3-etherate and a similar reaction was postulated to occur in the dealkylation step of (IV) in insects.[3]

The tritium-labelled substrates used in these studies were prepared by alumina-catalysed tritiation of 3-keto-4-ene-steroids.[4] The specific activities are shown in the Table.

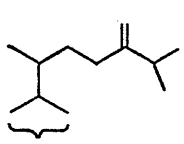
A solution of the [2,4-^{3}H]sterol in DMF was injected through the mouth into the 5th instar larvae of the silkworm, *Bombix mori*. The insects were sacrificed 24 h later.



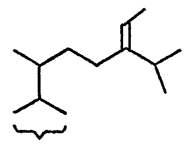
SCHEME

Cholesterol was isolated and purified. The incorporation into (I) from each substrate is shown in the Table.

In order to verify the transformation of (II) into (V), unlabelled (V) and 2 h later, [2,4-^{3}H](II) were given successively to *B. mori*. The insects were extracted 12 h later, and (V) as well as (I) were isolated. The incorporation of (I) from (II) was 1·6%. The epoxide (V) with specific activity of $9{\cdot}42 \times 10^3$ c.p.m./mg [incorporation from (II), 2·5%] was further converted into a 24,28-diol ($8{\cdot}24 \times 10^3$ c.p.m./mg), by treatment with H_2SO_4–H_2O, without significant loss of radioactivity.



(VI)



(VII)

These data suggest that (V)† is a probable intermediate in the conversion of (II) into (I). Thus, in conjunction with the previous findings,[5] we propose the Scheme as a main dealkylation route to (IV) in *B. mori*. A step-wise dealkylation route reminiscent of the reverse of phytosterol biosynthesis[2] [*e.g.* (IV) → (VII) → (VI) → (III) → (I)], seems to be of minor importance from the rather small incorporation of (VI) and (VII) into (I).‡

TABLE

Substrate (specific activity, μCi mg^{-1})	Incorporation into (I) (%)
Fucosterol-24,28-epoxide (V) (43)	15
Fucosterol (II) (230)	10
24-Methylenecholesterol (VI) (70)	3·2
Isofucosterol (VII) (43)	1·9

Dietary sterols are known to be essential for normal development and survival of *B. mori*.[6] We have investigated the nutritional effect of several sterols, which may be the possible candidates of dealkylation substrates. Thus, compounds (I)—(VII), when added, at 0·1%, to the diet satisfied the sterol requirement of *B. mori*, while insects fed with 28-oxo-, 24,28-dihydroxy-, and 24-hydroxy-28-oxositosterol and 24-oxo- and 24-hydroxy-cholesterol died during first instar. The latter observations probably exclude a dealkylation route analogous to the side chain cleavage of cholesterol or pregnenolone in vertebrates.

(*Received*, 19*th September* 1972; *Com.* 1606.)

† We have no information about C-24,28 stereochemistry of the endogenous epoxide(V). The substrate and carrier used in these works were synthesized from (II) by treatment with *m*-chloroperbenzoic acid, and therefore, they may be stereoisomeric mixtures.

‡ Compound (VI) could not be found as a de-ethylation intermediate of (IV) in locust[2b], while (VI) was identified as a demethylation intermediate in the conversion of campesterol into (I) in tobacco hornworm. J. A. Svoboda, M. J. Thompson, and W. E. Robbins, *Lipids*, 1972, **7**, 156.

[1] R. B. Clayton, *J. Lipid Res.*, 1964, **5**, 3.
[2] (a) J. A. Svoboda and W. E. Robbins, *Experientia*, 1968, **24**, 1131; J. A. Svoboda, M. J. Thompson, and W. E. Robbins, *Nature, New Biology*, 1971, **230**, 57; (b) J. P. Allais and M. Barbier, *Experientia*, 1971, **27**, 507.
[3] N. Ikekawa, M. Morisaki, H. Ohtaka, and Y. Chiyoda, *Chem. Comm.*, 1971, 1498.
[4] M. J. Thompson, O. W. Berngruber, and P. D. Klein, *Lipids*, 1971, **6**, 233.
[5] N. Ikekawa, M. Suzuki, M. Kobayashi, and K. Tsuda, *Chem. Pharm. Bull.*, 1966, **14**, 834.
[6] T. Ito, K. Kawashima, M. Nakahara, K. Nakanishi, and A. Terahara, *J. Insect Physiol.*, 1964, **10**, 225.