# Isolation of Fucosterol Epoxide from Larvae of the Silkworm, *Bombyx mori*

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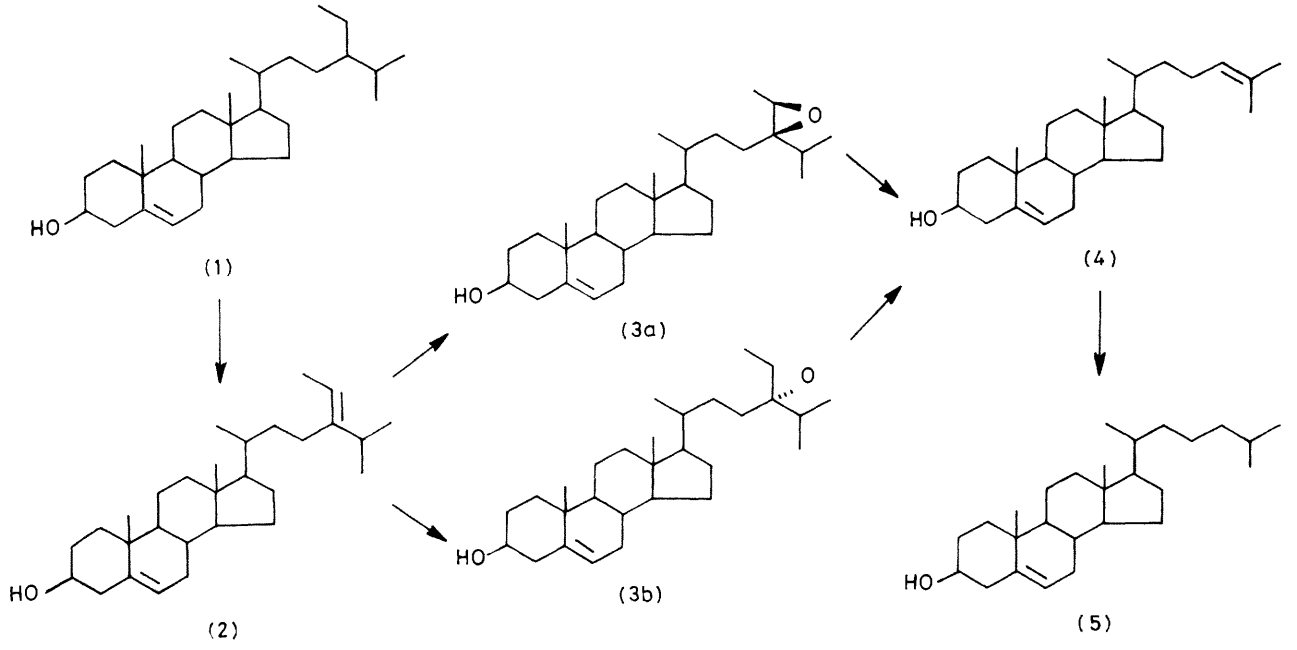
*Summary* Both isomers of (24*R*,28*R*)- and (24*S*,28*S*)-fucosterol epoxide, proposed as a intermediate in the conversion of sitosterol into cholesterol in the silkworm *Bombyx mori*, were isolated from the larvae.

SINCE we proposed[1] the following biosynthetic pathway from sitosterol (**1**) to cholesterol (**5**) in the silkworm *Bombyx mori:* (**1**) → fucosterol (**2**) → fucosterol epoxide (**3**) → desmosterol (**4**) → (**5**), many results[2] supporting this pathway have been reported. However, the crucial demon-

stration of this bioconversion would be the isolation of fucosterol epoxide (**3**) from the insect itself [3] We now report the identification of both stereoisomers of fucosterol epoxide (**3a** and **b**) from the fifth instar larvae of the silkworm




In preliminary investigations, the epoxide fraction was converted into the hydroxy derivatives which were detected by g l c–mass spectrometry † Thus, 24-hydroxysitosterol, after treatment with excess of $LiAlH_4$, and 24,28-dihydroxysitosterol, after treatment with $H_2SO_4$, were detected by monitoring of the ions at *m/e* 531 and 545, respectively, indicating the presence of the epoxide in the larva The isolation of the epoxide was completed by the following procedure

Fifth instar larvae (200 larvae) were homogenized with $CHCl_3$–MeOH, and the solution was applied to a column of silica gel The sterol fraction was saponified with 5% KOH–MeOH, and the unsaponifiable neutral fraction was chromatographed again on silica gel The fraction eluted with benzene–ethyl acetate (50 : 1) was benzoylated, and the benzoate was purified by column chromatography on silica gel and then by preparative t l c with benzene–ethyl acetate (50 : 1, $R_f$ 0 7) The benzoate (3 mg) was subjected to h p l c using Zorbax ODS The fraction corresponding to a small peak with the retention time of fucosterol epoxide benzoate was collected, and subjected again to h p l c using Zorbax SIL The retention times of the two peaks coincided exactly with those of the benzoates of (24*R*,28*R*)-(**3a**) and (24*S*,28*S*)-fucosterol epoxide (**3b**) [4] The mass spectra of both fractions, *m/e* 532 ($M^+$), 489 ($M$ – $Pr^iO$), 410 ($M$ – $PhCO_2H$), 395 ($M$ – $PhCO_2H$ – Me), 366 ($M$ – $PhCO_2H$ – MeCHO), 252, 213, and 145, were almost identical with that of authentic fucosterol epoxide benzoate For further structural proof, each fraction was reduced with $LiAlH_4$ and the trimethylsilyl derivative of the product was analysed by g l c–mass spectrometry, which showed a single peak for each fraction The retention time and mass spectrum were identical with those of the trimethylsilyl derivative of 24-hydroxysitosterol

Since the h p l c technique can separate isofucosterol epoxide from fucosterol epoxide,[4] the chromatogram of the material obtained from the silkworm larvae indicates that they do not contain isofucosterol epoxide From the peak areas, the amounts of the epoxides were roughly estimated as 100 ng/larva for the (24*R*,28*R*)-epoxide (**3a**) and 300 ng/larva for the (24*S*,28*S*)-isomer (**3b**) To clarify whether the epoxide exists in the free or the conjugate form, the sterol fraction (488 mg) obtained from 300 of the fifth instar larvae was benzoylated without saponification and subjected to silica gel column chromatographic, t l c , and then h p l c purification The chromatogram showed two peaks corresponding to (**3a**) and (**3b**), the areas of which indicated the presence of *ca* 70 and 180 ng/larva, respectively Thus, the epoxides appear to be present mainly in their free form

The isolation of fucosterol epoxide corroborates the intermediacy of the epoxide in the bioconversion of sitosterol into cholesterol in the insect We showed previously[5] that the stereospecificity in the formation and the metabolism of the epoxide is not strict, and that both isomers may be the intermediates in sitosterol dealkylation This

† A Shimadzu LKB-9000S g l c–m s system with 1 5% OV-17 (1 m × 3 mm i d , 280 °C) was used for the trimethylsilyl derivative

conclusion is strongly supported by the present results which indicate that both the stereoisomers (**3a**) and (**3b**) are present in the silkworm. It is possible, from the roughly estimated amounts of the epoxides, that both isomers are formed in equal amounts, at least from the evidence of the *in vitro* study,[5] and the isomer (**3a**) is converted into desmosterol (**4**) faster than the isomer (**3b**).

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