Stereochemistry of the Hydrogen Addition to C-25 of Desmosterol by Sterol-Δ^{24}-reductase of the Silkworm, *Bombyx mori*

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The C-25 stereochemistry of the reaction catalysed by sterol-Δ^{24}-reductase from the insect, *B. mori*, has been investigated by the use of ^{13}C NMR spectroscopy; incubation of isopropylidene (*E*)-methyl-^{13}C labelled desmosterol afforded cholesterol with the label at *pro-R*-methyl of the isopropyl group, and the results indicated the C-25 hydrogen was introduced stereospecifically from the *si*-face of the double bond.

Sterol-Δ^{24}-reductase is an essential mammal enzyme; it catalyses the reduction of 24(25)-unsaturated steroids, such as lanosterol and desmosterol.[1] The stereochemistry involved was elucidated by use of radiolabelled compounds, *i.e.* addition of hydrogen takes place in a *cis* fashion in which hydrogen from water is introduced to the C-24 position from the *si*-face[2] and the 4-*pro-R*-hydrogen (4-H_B) of NADPH is transferred to the C-25 position from the *si*-face.[3]

Similarly, to compensate for the absence of *de novo* sterol biosynthesis, insects possess sterol-Δ^{24}-reductase, which is involved in the last step of the dealkylation of plant sterols such as sitosterol (Scheme 1).[4] Little is known from comparative studies of the enzymes of mammalian and insect origin.[5] We have systematically studied the mechanism of the sterol dealkylation in insects, and have now elucidated the C-25 stereochemistry of the reduction by sterol-Δ^{24}-reductase of the silkworm, *B. mori* by ^{13}C NMR spectroscopy.

Preparation of the isopropylidene (*E*)-methyl-^{13}C-labelled desmosterol was reported previously.[6] This particular desmosterol was enriched to more than 98% ^{13}C [85% of the label resided at (*E*)-methyl and 15% at (*Z*)-methyl].[6,7] A cell-free system (1500 g supernatant fraction in Bücher's medium, pH 7.4, protein concentration 10 mg ml^{-1}) was prepared from the guts of fifth instar larvae (on day six) of the silkworm, *B. mori*, according to the published method.[8] The ^{13}C-labelled demosterol (300 μg) was incubated with the cell-free preparation (5 ml) in the presence of NADPH (4 mg) for 3 h at 30 °C aerobically.

The incubation mixture was repeatedly extracted with ethyl acetate and the concentrated extract was separated by preparative TLC on a silica gel plate to afford the sterol fraction. The ^{13}C NMR spectrum of the fraction is shown in Fig. 1. Since the ^{13}C enrichment of the substrate was so high that the natural abundance signals of various organic materials in the fraction could not be seen in the spectrum, this allowed us to focus only on the substrate and any products.† Along with the signal (c) of the substrate at δ 25.72, an intense signal (a) is observed at 22.56, which is attributable to the *pro-R*-methyl (C-26[9]) of cholesterol. Further the weak signal at 22.82 is ascribable to *pro-S*-methyl (C-27[9]). The chemical shifts of the *pro-R*- and *pro-S*-methyl groups of cholesterol were previously established.[9,10]

† The very weak, but obvious signals corresponding to C-11, C-15 and C-19 are due mainly to sitosterol, which is a major sterol contained in the cell-free preparation.

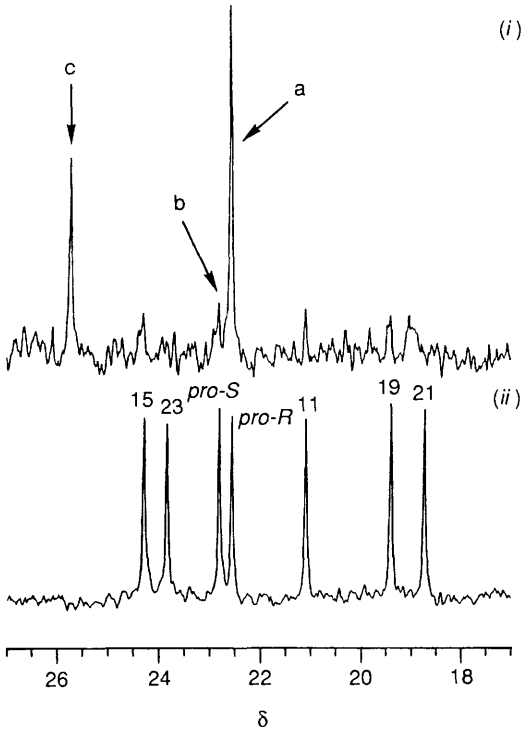
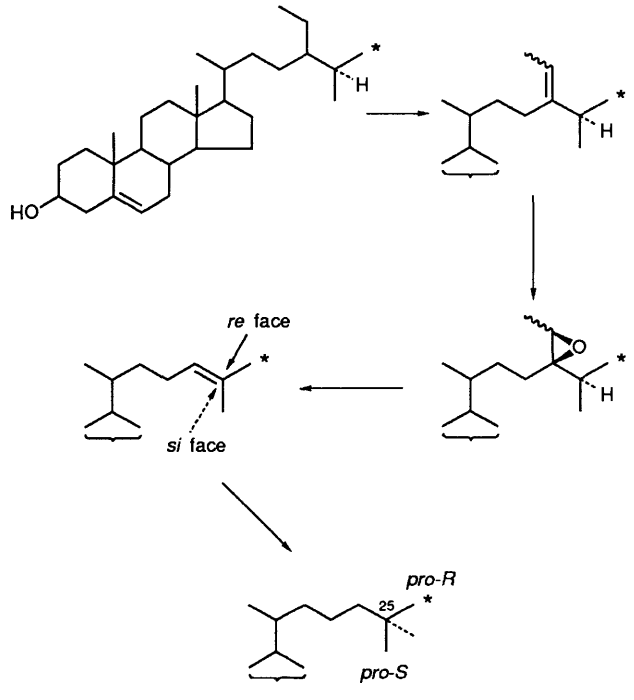


Fig. 1 ^{13}C NMR (125 MHz, $CDCl_3$) spectra of the incubation product (sterol fraction) (*i*) and non-labelled cholesterol (*ii*). Signals a, b and c are due to *pro-R*- and *pro-S*-methyl groups of cholesterol and (*E*)-methyl group of desmosterol, respectively.



Scheme 1 Metabolic fate of the diastereotopic methyl groups on the C-25 prochiral centre in the conversion of sitosterol into cholesterol in *B. mori*. The asterisk indicates metabolically correlated methyl group.

The formation of cholesterol was also confirmed by GC–MS analysis of an aliquot of the sterol fraction in the form of the trimethylsilyl ether. The abundance of ^{13}C-labelled cholesterol to endogenous cholesterol was calculated to be *ca.* 1 : 3 based on the intensity of the peaks at *m/z* 461 (7%), 460 (25%), 459 (70%) and 458 (100%, M^+ of the non-labelled ether).‡

The ratio (*ca.* 7 : 1) of the *pro-R*- and *pro-S*-methyl groups of cholesterol was almost the same as that of the (*E*)- and (*Z*)-methyl groups of the precursor (see above). As a consequence, it now appears that the reduction catalysed by sterol-Δ^{24}-reductase of *B. mori* is stereospecific, that is, the (*E*)- and (*Z*)-methyl groups of desmosterol become the *pro-R*- and *pro-S*-methyl groups of cholesterol, respectively. The hydrogen atom at C-25 of cholesterol is introduced from the *si*-face of the double bond. The stereochemical course observed in the insect turned out to be identical with that found in the formation of cholesterol from lanosterol in rats.

It should also be emphasized that the present method, which took advantage of ^{13}C NMR spectroscopy, appears to be much simpler and straightforward than the one using radioisotopes.

We have recently demonstrated the stereochemical course of the conversion of fucosterol epoxide and isofucosterol epoxide into cholesterol in *B. mori*.[7,11] By combining the present as well as the previous results, the metabolic fate of the diastereotopic methyl group during the dealkylation in *B. mori* can be summarised as depicted in the Scheme 1.§

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‡ It is unnecessary to take into account the amount of endogenous desmosterol, which is less than a few % of the endogenous cholesterol.

§ The C-25 stereocentre is assumed to be retained in the first two steps.