Sterol Metabolism in Insects: Dealkylation of Phytosterol to Cholesterol

NOBUO IKEKAWA,*,† MASUO MORISAKI,‡ AND YOSHINORI FUJIMOTO§

College of Science and Engineering, Iwaki Meisei University, Iwaki, Fukushima 970, Japan, Kyoritsu College of Pharmacy, Minato-ku, Tokyo 105, Japan, and Department of Chemistry, Tokyo Institute of Technology, Meguro, Tokyo 152, Japan

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Cholesterol is ubiquitous in the animal kingdom, serving as a cell membrane component and as a precursor of steroid hormones such as cortisol in mammals and ecdysone in insects. Mammals obtain cholesterol either by dietary absorption or by biosynthesis from mevalonate. Since insects have no capacity for de novo sterol synthesis, they rely exclusively on exogenous sources, e.g., phytosterols such as sitosterol, which is converted by insects into the requisite cholesterol (Figure 1). This is carried out by carbon–carbon bond cleavage of the alkyl group at the C-24 position of phytosterols, as described herein.

Carbon–carbon bond cleavage reactions occur widely in biochemical processes as exemplified at various stages of steroid metabolism,[1] including (1) cleavage of 1,2-glycol to give two carbonyl compounds, e.g., side chain cleavage of cholesterol catalyzed by adrenocortical cytochrome P-450;[2] (2) deformylation with a concomitant loss of β-hydrogen, e.g., 14-demethylation of lanosterol;[3] (3) decarboxylation of β-oxo carboxylic acid, e.g., demethylation of 4,4-dimethylsterols;[4] (4) thiolytic cleavage of β-oxo carboxylic acid derivatives, e.g., C-24,25 bond cleavage during bile acid biosynthesis;[5] and (5) photolytic cleavage of the C-9,10 bond of a 5,7-diene compound to yield a vitamin D derivative.[6] In insects, however, another type of C–C bond cleavage occurs, which is effected through a fragmentation reaction of an epoxide. This is the subject of the present Account.[7]

Chemical Background

The action of acids on epoxides can produce a wide range of ring-opening and rearrangement reactions.[8] In the course of our studies on marine algal sterols and the chemistry of steroid side chains, the 24,28-epoxide of fucosterol acetate was treated with boron trifluoride etherate. To our surprise, the formation of a nonpolar product (35% yield), together with a ketone (46%) and an aldehyde (12%), was observed (Figure 2, eq 1). The unexpected product was subsequently characterized as desmosterol acetate, in which the C-28 and C-29 carbons have been excised.[9]

The unique fragmentation reaction, which we first published in 1971, appeared to be mechanistically related to phytosterol dealkylation in insects and prompted us to undertake subsequent studies on the mechanism of phytosterol dealkylation in the silkworm, *Bombyx mori*.

The formation of the three products can be rationalized as shown in the scheme (Figure 2). Thus pathway a yields desmosterol acetate and acetaldehyde, pathway b yields the 28-ketone, and pathway c yields the 24-methyl-24-formyl product.[9] Such fragmentation reactions are infrequent and two additional examples are given in Figure 2; eqs 2[10] and 3.[11] It seems that tri- or tetrasubstituted epoxides which bear at least one methine hydrogen on the adjacent substituent may eventually undergo such fragmentation reactions.

Mechanism of Phytosterol Dealkylation

Insects, as described above, cannot synthesize sterols de novo but require dietary sterol for their normal growth, development, and reproduction. This sterol

Nobuo Ikekawa, born in 1926, received his B.Sc. degree in 1951 and Ph.D. from the University of Tokyo in 1959. In 1969, he joined Tokyo Institute of Technology (TIT), where he served as a professor in the Department of Chemistry. In 1987, he moved to Iwaki Meisei University. His research has focused on the chemistry and biochemistry of steroids and other natural products, including insect sterols, vitamin D metabolites, and brassinosteroids. He is the author and coauthor of more than 400 publications and is now a professor emeritus of TIT.

Masuo Morisaki was born in 1937. He received his B.Sc. degree from Chiba University in 1960 and Ph.D. from the University of Tokyo in 1968 before becoming a research associate at TIT during 1969–1984. He spent one year (1970–1971) with Professor K. Bloch at Harvard University as a postdoctoral fellow and moved in 1984 to Kyoritsu College of Pharmacy, where he is now a professor in medicinal chemistry. His research field is the bioorganic and medicinal chemistry of steroids.

Yoshinori Fujimoto was born in 1949. After receiving B.Sc. and M.Sc. degrees from TIT, he obtained his Ph.D. (1979) in chemistry at TIT. He then spent a postdoctoral period with Professor C. J. Sih at the University of Wisconsin—Madison and has been at TIT since 1982, where he is now an associate professor in the Department of Chemistry. His research interests have focused on the bioorganic chemistry of natural products including steroids.

† Iwaki Meisei University.
‡ Kyoritsu College of Pharmacy.
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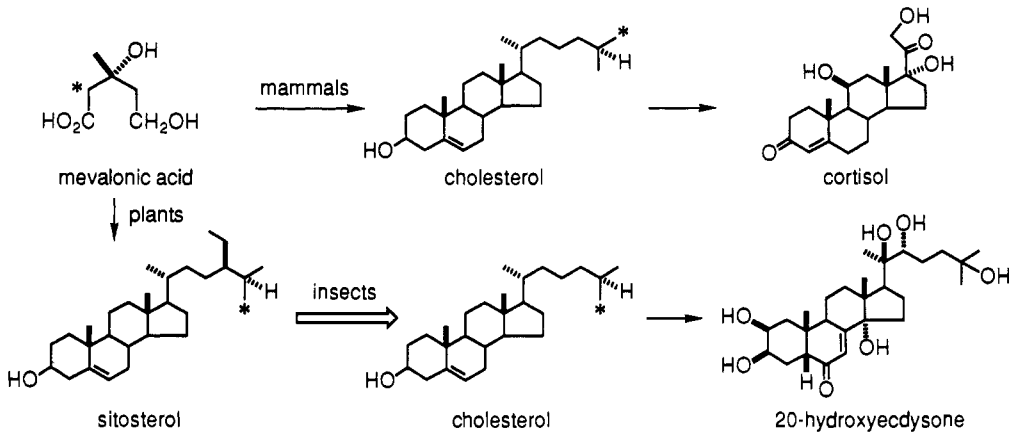

Figure 1. Steroid metabolism in insects and mammals. Asterisks indicate the diastereotopic methyl group derived from C-2 of mevalonate.

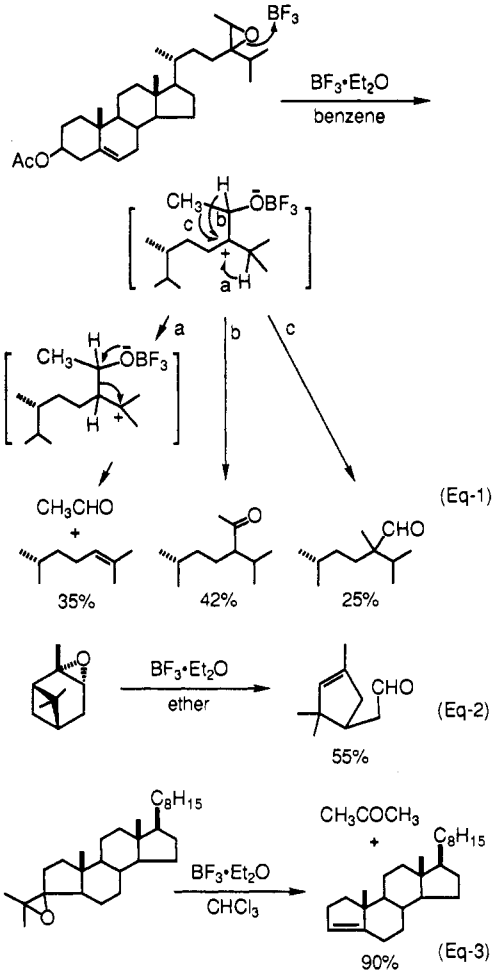

Figure 2. BF_3-catalyzed carbon–carbon bond cleavage reactions of epoxides.

requirement is in most cases satisfied by cholesterol (1), which is the principal sterol in most insects. Carnivorous insects like houseflies take cholesterol as such from their diets. In phytophagous insects, however, the requirement may also be satisfied by C-24-alkylated plant sterols such as sitosterol (13), campesterol (7), and stigmasterol (15), which are metabolized to cholesterol (1). Thus 24-dealkylation is one of the essential metabolic processes in phytophagous insects. Since the first rigorous demonstration of the dealkylative conversion of ergosterol into 22-dehydrocholesterol (33) in the German cockroach *Blattela germanica*,[12] several reports have appeared on the conversion of phytosterols into cholesterol in a variety of insects, including *B. mori*.[13] In the tobacco hornworm *Manduca sexta*, fucosterol (19) and desmosterol (2) were identified as intermediates in sitosterol dealkylation.[14]

Our observation that fucosterol 24,28-epoxide (17/18) affords desmosterol (2) upon treatment with boron trifluoride etherate (vide supra) led us to propose that the epoxide 17/18 is a key intermediate in the conversion of sitosterol (13) to cholesterol (1) in insects (Figure 3).[9] This was subsequently verified in *B. mori*. When [2,4-^{3}H]fucosterol epoxide (17/18) was ingested by silkworm larvae, it was converted to cholesterol (1) in considerable yield. Similarly, following administration of [2,4-^{3}H]fucosterol (19), tritiated epoxide 17/18 was obtained.[15] The tritium of [25-^{3}H]sitosterol migrated to the C-24 position during its conversion to demosterol (2).[16] Epoxide 17/18 completely satisfied the silkworm sterol requirement[17] and was isolated from *B. mori* larvae reared on mulberry leaves, its natural diet.[18] Results from *Tenebrio molitor*[19] and *Locusta migratoria*[20] support the biogenetic pathway shown in Figure 3.

In the dealkylation of campesterol (7), 24-methylenecholesterol (11) has been identified as an intermediate in *M. sexta*.[21] An intermediary role of the 24,28-epoxide 38 has been demonstrated in the silkworm using [24-^{2}H]-, [25-^{2}H]-, and [23,23,25-^{2}H]campesterol and [23,23,25-^{2}H$_3$]-24-methylenecholesterol. The results of GC–MS analysis of the metabolically produced cholesterol (1) and desmosterol (2) from these deuterated substrates suggested that the C-25 deuterium atom migrated to the C-24 position of desmosterol (2) during

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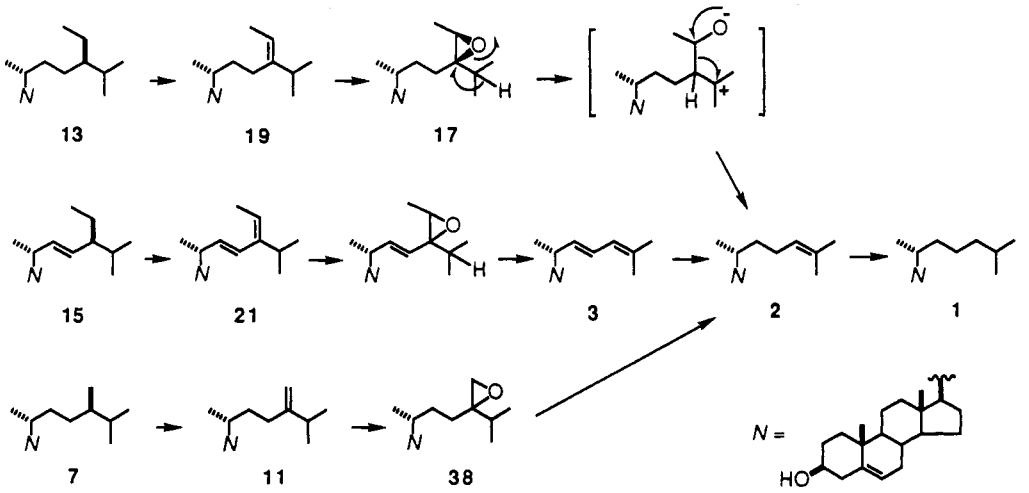

Figure 3. Mechanism of C-24 dealkylation of phytosterols in insects.

dealkylation.[22] Similar results were also obtained in *T. molitor* using [23,23,25-$^3H_3$]-24-methylenecholesterol.[23]

The same methodology was applied to examine an intermediary role of 24,28-epoxide in stigmasterol dealkylation. Samples of [23-^{2}H]-, [24-^{2}H]-, and [25-^{2}H]stigmasterol were fed to *B. mori*, and both cholesterol and desmosterol extracted from the insects were found to contain one, zero, and one deuterium atom, respectively. 5,22,24(28)-Trienes **21** and **22** satisfied the sterol requirement of the silkworm. 5,22,24-Triene **3** and desmosterol (**2**), but not 22-dehydrocholesterol (**33**), were identified in significant amounts from insects ingesting stigmasterol (**15**) or trienes **21** and **22**.[24] These results support the generality of the dealkylation mechanism depicted in Figure 3, including campesterol and stigmasterol.

Sterol Structure Requirement of *Bombyx mori*

B. mori can grow and develop on a semisynthetic diet containing cholesterol or phytosterol at 0.1–0.5%.[25] In place of cholesterol and phytosterol, a variety of sterols were added to the diet, and their nutritional effects were evaluated. Sterols are classified as "effective" if they behave in the same manner as cholesterol and phytosterol, "partially effective" if *B. mori* survived through the early instars but with a slower growing rate, and "ineffective" if all larvae died in the first instar (Figure 4).[17] As described above, dealkylation intermediates such as **2**, **3**, **11**, **17**, **18**, **19**, and **21** belong to the "effective" group. The fact that the oxygenated sterols **52–56** are "ineffective" seems to exclude an alternative pathway of sitosterol/stigmasterol dealkylation, **13** → **53** → **56** → **55** → **2** → **1** and **13** → **52** → **54** → **56** → **55** → **2** → **1**, reminiscent of the side chain cleavage of cholesterol or pregenolone in vertebrates. Similarly, an oxidative cleavage of campesterol, i.e., **7** → **58** → **59** → **1**, is also improbable, since these sterols are classified as "ineffective". Notably the 24,28-epoxide **38**, which is a postulated intermediate of campesterol dealkylation, supported the growth of *B. mori* only inadequately. It may be considered that most of the ineffective sterols described above are relatively hydrophilic in nature and do not pass the cell membrane barrier to the site of dealkylation. Further, when such sterols are ingested in the large amounts used for nutritional experiments, they may deleteriously affect normal insect physiology.

A series of cholesterol analogs was examined for nutritional effects on *B. mori* larvae, and the results are included in Figure 4.[26-28] Slight deviations from the cholesterol side chain, as shown in **25–30** and **47–49**, induced pronounced growth-retarding effects, suggesting an important functional role of the isooctane side chain structure of cholesterol. The special importance of the C-20 stereochemistry is indicated by the complete ineffectiveness of 20-isocholesterol (**51**) and (*Z*)-20(22)-dehydrocholesterol (**50**). The silkworm can tolerate certain modifications of the sterol nucleus, as was indicated by the observation that 6-ene **4**, 7-ene **5**, and 5,7-diene **6** were able to replace cholesterol as sterol sources for *B. mori*; fairly good growth was also obtained with 19-norcholesterol (**45**), 5α-cholestanol (**42**), and certain cholesterol and cholestadienol isomers (**43**, **44**, etc.). In contrast, 19,19-difluorocholesterol (**63**), 5β-cholestanol (**65**), and 4,4-dimethylcholesterol (**64**) are completely ineffective. The serious deleterious effect of the introduction of the 4,4-dimethyl group was noticed in studies using the hide-beetle *Dermetes vulpinus*,[29] while the effect of an "extra" 14α-methyl group is less pronounced since 14-methylcholesterol (**46**) is classified as a "partially effective" sterol.[27]

Insect sterol analysis by GC–MS revealed that most of the effective or partially effective sterols listed in Figure 4 were utilized by *B. mori* as such, without significant metabolic conversion to other sterols (except side chain dealkylation/hydrogenation).

Inhibitors of Sterol Metabolism in Insects

Inhibitors of sterol metabolism have proven to be valuable tools for studying phytosterol metabolism. For

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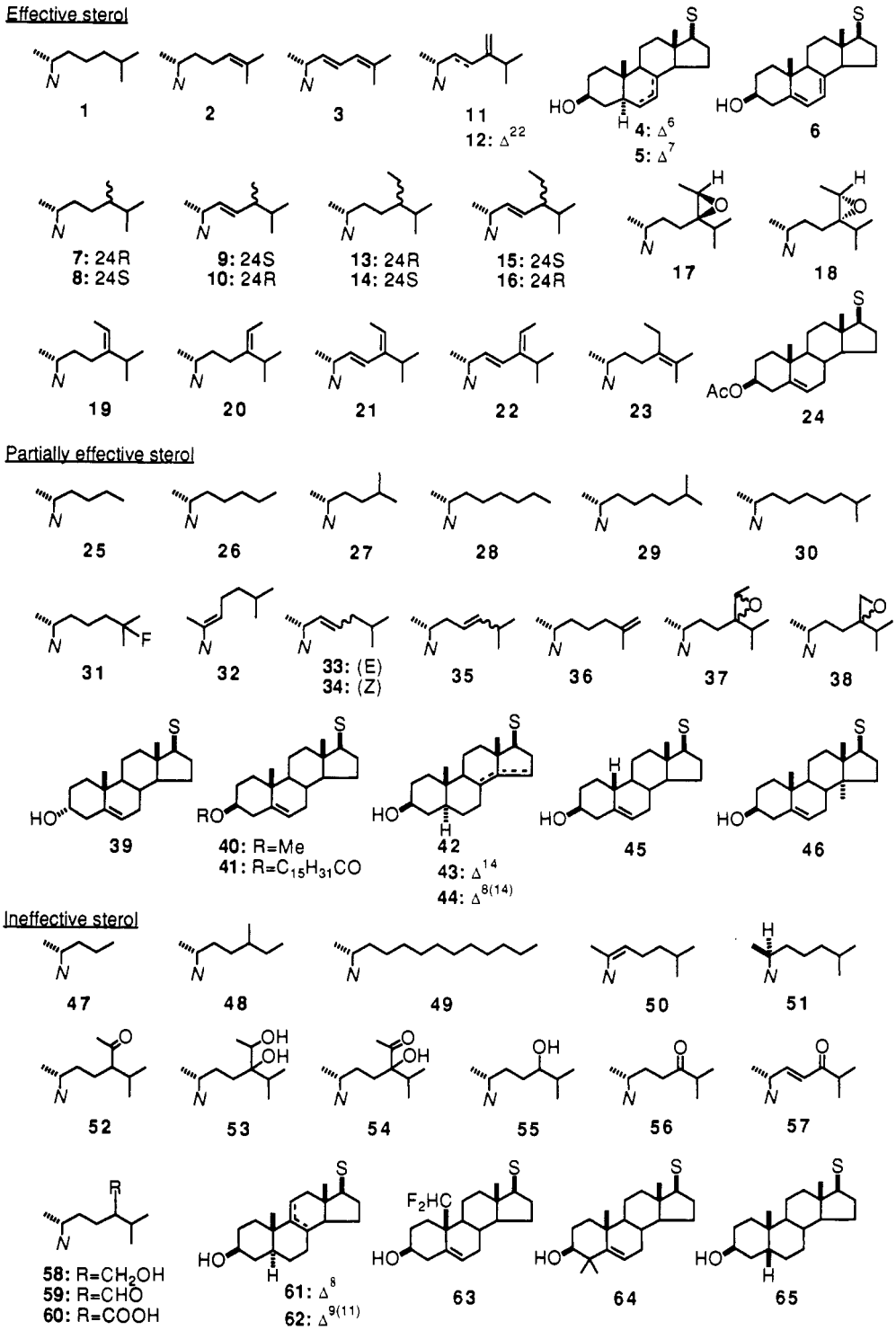

Figure 4. Classification of sterols based on their nutritional effects on *B. mori*. *N* is as defined in Figure 3; S means isooctane side chain.

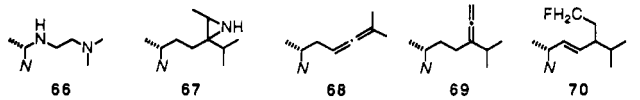

Figure 5. Inhibitors of sterol metabolism in insects. *N* is as defined in Figure 3.

example, 22,25-diazacholesterol (**66**) (Figure 5) is an inhibitor of Δ^{24}-sterol reductase and was used for identification of desmosterol (**2**) and cholesta-5,22,24-trien-3β-ol (**3**) as intermediates in phytosterol dealkylation.[30] Subsequently other azasteroids and some nonsteroidal amines were found to block the conversion of 24-alkylsterols to cholesterol and/or disrupt molting and development in insects.[31] We postulated that sterol analogs of dealkylation intermediates would be inhibitors of insect sterol metabolism, and therefore we synthesized 24,28-iminositosterol (**67**),[32] cholesta-5,23,-24-trien-3β-ol (**68**), and stigmasta-5,24(28),28-trien-3β-ol (**69**).[33]

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When imine **67** was administered in the silkworm diet in combination with sitosterol or cholesterol, the growth and development of *B. mori* were markedly retarded. Indeed the imine inhibited the in vitro conversion of tritiated fucosterol epoxide to desmosterol, most likely at the epoxide level.[34] Apparently the imine inhibits not only the epoxidative dealkylation, as evidenced by the fact that dietary cholesterol did not prevent the inhibitory effect.

Allene **68** seemed to exert little effect on sitosterol dealkylation because the sterol profile in the silkworm fed on the allene in combination with sitosterol was essentially the same as that in the control.[35]

The other allene (**69**) has far more interesting properties. When *B. mori* was reared on fucosterol epoxide, desmosterol, or cholesterol, the addition of allene **69** to the diet at the same concentration as that of nutritional sterol did not inhibit insect growth and development. In contrast, when the nutritional sterol was sitosterol or fucosterol, marked growth retardation was observed. In agreement with these results, allene **69** induced accumulation of sitosterol and fucosterol when these were used as the dietary sterols. The results strongly suggest that allene **69** is a highly specific inhibitor for the steps involving fucosterol, that is, for the conversion of sitosterol to fucosterol and/or fucosterol to the epoxide.[35] It is interesting to note that this same allene (**69**) was identified in the sterol composition of the sponge *Callyspongia diffusa*.[36]

Several sterols fluorinated in their side chains have been synthesized and tested for their inhibitory actions on the growth of tobacco hornworm, *M. sexta*.[37] 29-Fluorostigmasterol (**70**) and 29-fluorositosterol gave significant impairment of growth and development of the larvae. Abnormal development caused by the 29-fluorosterols is similar to that seen in larvae fed on sodium fluoroacetate or ω-fluoro fatty acid. [2-^{3}H]-*erythro*-2-Fluorocitrate was identified in *M. sexta* ingesting [29-^{3}H]-29-fluorostigmasterol (**70**).[38] These results suggest that 29-fluorinated sterol metabolized to fluoroacetate, causing the toxicity. Neither toxicity nor abnormal growth was found with various monofluorocholesterols, such as 25-fluorocholesterol (**31**) and 26-fluorocholesterol, incorporated into diets fed to *M. sexta*. The 25-fluorocholesterol (**31**) is a "partially effective" sterol, and desmosterol and cholesterol were identified in *B. mori* fed 25-fluorocholesterol.[28]

An extension of these studies on dealkylation inhibitors should lead, we hope, to discovery of highly specific insecticides that are nontoxic to plants as well as humans.

Stereochemical Aspects of Phytosterol Dealkylation

Stereoselectivity of Phytosterol Dealkylation. Certain structures of C-24 epimeric pairs of 24-alkylsterols (**7–10, 13–16**) are included in Figure 4. All of these sterols are classified as "effective sterols", suggesting that *B. mori* larvae are able to convert these sterols into cholesterol to the extent required for normal growth and development. This was verified by insect sterol analysis.[39] Nonselective dealkylation of phytosterols can be seen in many other insects. For example, in feeding experiments using *T. molitor* larvae, sitosterol (**13**) and clionasterol (**14**) were incorporated into fucosterol (**19**) with equal efficiency, whereas clionasterol was metabolized into isofucosterol (**20**) less readily than sitosterol.[40] The lack of stereospecificity in the first step of dealkylation is noteworthy. A direct dehydrogenation mechanism is proposed rather than a hydroxylation–dehydration sequence, since negligible conversions of the hydroxy intermediates were observed.[41] We speculate that *B. mori* has a single dehydrogenase enzyme, which is able to convert a variety of phytosterols to 24(28)-olefinic intermediates.

Lack of stereospecificity was also observed with 24(28)-olefinic sterols. As previously described for compounds **19–22**, these (*Z*)- and (*E*)-isomers are equally utilized by *B. mori*.[17,24] Both fucosterol (**19**) and isofucosterol (**20**) were identified in the sterol fraction from *B. mori* reared on mulberry leaves.[42]

Several lines of evidence have been reported on the stereochemical aspects of 24,28-epoxides of fucosterol and isofucosterol. Oral administration of [^{3}H]-(24*R*,28*R*)- and (24*S*,28*S*)-epoxides (**17** and **18**) to *B. mori* larvae afforded almost equal amounts of labeled cholesterol.[43] Furthermore, the two epoxides fully supported the growth and development of *B. mori*. In contrast, a 1:1 mixture of (24*R*,28*S*)- and (24*S*,28*R*)-isomers (**37**) only partially supported the growth of the larvae.[44] In addition, a mixture of (24*R*,28*R*)- and (24*S*,28*S*)-epoxides (**17** and **18**) was found in *B. mori* larvae reared on mulberry leaves. In this mixture, neither the (24*R*,28*S*)- nor the (24*S*,28*R*)-isomer (**37a** and **37b**; **a** and **b** refer to (24*R*,28*S*)- and (24*S*,28*R*)-epoxides, respectively) was detected.[18]

In contrast, a highly selective conversion of (24*R*)-epoxides was observed in in vitro experimentation. Thus, a cell-free preparation from the gut tissue of *B. mori* converted only (24*R*,28*R*)-epoxide **17** and (24*R*,28*S*)-epoxide **37a**, but not the (24*S*)-isomers **18** and **37b**, into desmosterol and/or cholesterol.[44–46] Given this inconsistency between in vivo and in vitro results, neither set of experiments permits us to answer the question of which stereoisomer(s) may be a true intermediate in sitosterol dealkylation in *B. mori*. We

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can nevertheless speculate on the importance of (24*R*,28*R*)-isomer **17** as an intermediate of sitosterol dealkylation.

A similar 24*R* preference has been reported in cell-free experiments using *M. sexta*, where (24*R*,28*R*)-epoxide **17**, but not (24*S*,28*S*)-isomer **18**, was converted into desmosterol and cholesterol.[47] A somewhat different stereochemical preference was reported when *T. molitor* larvae were used. The insects metabolized (24*R*,28*S*)-epoxide **37a** to cholesterol more efficiently than (24*S*,28*R*)-isomer **37b**, whereas (24*R*,28*R*)- and (24*S*,28*S*)-epoxides **17** and **18** were equally incorporated into cholesterol.[48]

As described above, the 24,28-epoxide (**38**) of 24-methylenecholesterol (**11**) is proposed as an intermediate of campesterol (**7**) dealkylation. In experiments using a cell-free preparation from *B. mori*, a 1:1 mixture of (24*R*)- and (24*S*)-epoxides (**38a** and **38b**; **a** and **b** refer to 24*R* and 24*S* stereochemistry, respectively) was converted to desmosterol only half as effectively as (24*R*,28*R*)-fucosterol epoxide (**17**),[49] suggesting that the (24*R*)-isomer may be a more likely substrate for conversion by the epoxide lyase. The data supporting this idea was published using *T. molitor* larvae, which metabolized only (24*R*)-epoxide **38a**, but not (24*S*)-isomer **38b**, into cholesterol.[50]

Stereochemical Course in the Fragmentation of 24,28-Epoxide. As described above, the enzymatic transformation of 24,28-epoxide to desmosterol involves hydrogen migration from C-25 to C-24. This implies that an electron-deficient species forms initially at C-24 and then at the C-25 position, unless the whole sequence of the rearrangement proceeds in a highly concerted manner. Tracking of the C-25 prochirality in the enzymatic reaction would provide information on the mechanism of this enzyme reaction, e.g., orientation and stability of these electron-deficient species, in particular, the C-25 electron-deficient intermediate.

The fate of the diastereotopic methyl groups of 24,28-epoxides was conveniently traced by the use of ^{13}C-labeled compounds. *pro-R*-Me- and *pro-S*-Me ^{13}C-labeled fucosterol (24*R*,28*R*)-epoxides[51] were incubated with a cell-free preparation from *B. mori*, and the sterol fraction of the product was analyzed by ^{13}C-NMR (Figure 6). The results clearly indicate that the enzyme reaction is stereospecific, with the *pro-S* and *pro-R* methyl groups of both epoxides turning stereospecifically into (*Z*)- and (*E*)-methyl groups, respectively, of desmosterol. Incubation of the ^{13}C-labeled isofucosterol (24*R*,28*S*)-epoxides afforded the same stereochemical results.[52,53]

A most likely implication of these findings is depicted in Figure 7. When the epoxide interacts with the lyase enzyme, the isopropyl group seems to be oriented in

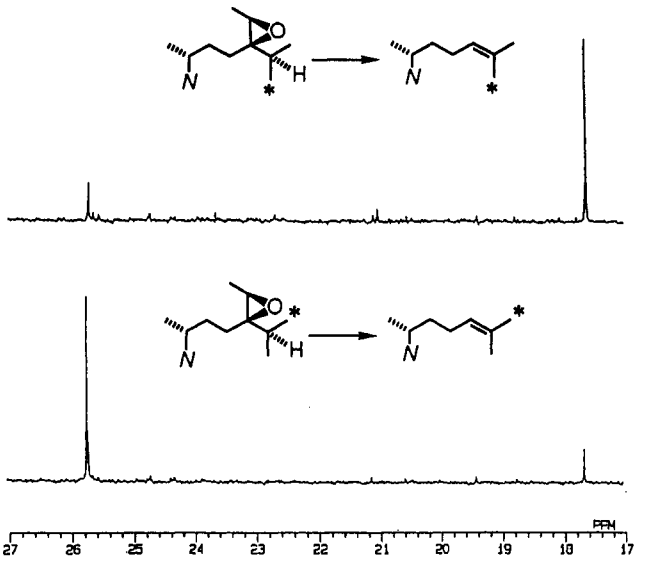

Figure 6. ^{13}C-NMR spectra of the sterol fraction obtained by incubation of *pro-S*-methyl (upper) and *pro-R*-methyl (lower) ^{13}C-labeled fucosterol (24*R*,28*R*)-epoxides. (*Z*)- and (*E*)-methyl signals of desmosterol are at δ 17.6 and 25.7, respectively. Asterisks indicate the ^{13}C label. *pro-S*-Methyl ^{13}C-labeled fucosterol (24*R*,28*R*)-epoxide is labeled 85% at *pro-S*-methyl and 15% at *pro-R*-methyl, and *pro-R*-methyl ^{13}C-labeled epoxide is labeled 85% at *pro-R*-methyl and 15% at *pro-S*-methyl.

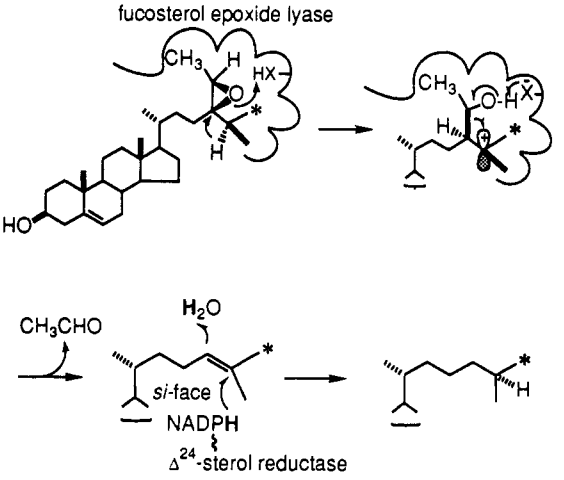

Figure 7. Proposed mechanism of enzymatic conversion of fucosterol epoxide to cholesterol. Asterisks indicate the metabolically correlated diastereotopic methyl group.

such a conformation that the C-25 hydrogen is antiperiplanar to the C-24–oxygen bond, which can facilitate a migration of the C-25 hydrogen to the C-24 position with an S_N2-type inversion at the C-24 center. The resulting C-25 electron-deficient species may eliminate acetaldehyde to form the 24(25) double bond without rotation of the C-24/C-25 single bond. It should be noted that the orientation of the C-28 methyl group does not alter the fate of the diastereotopic methyl groups. In these studies, the advantage of the use of the ^{13}C label coupled with ^{13}C-NMR spectroscopy should be emphasized.

In sharp contrast with the enzymatic fragmentation reaction of the 24,28-epoxide mentioned above, BF_3-catalyzed reaction of the benzoate of the same epoxide was found to proceed in a nonstereoselective manner (Figure 8).[54] Thus, when the *pro-S*-methyl (**71**) or *pro-R*-methyl (**72**) ^{13}C-labeled (24*R*,28*R*)-fucosterol epoxide 3-benzoate was treated with boron trifluoride etherate,

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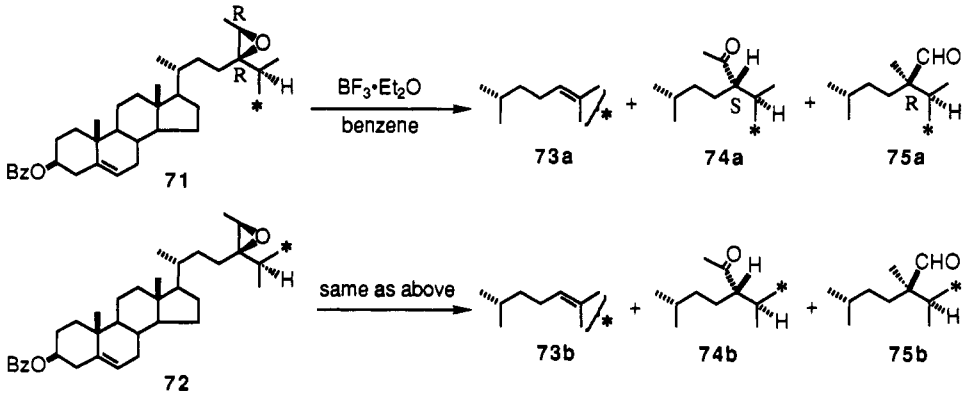

Figure 8. Stereochemical course of the BF_3-catalyzed rearrangement of fucosterol 24,28-epoxide benzoate. Asterisks indicate the ^{13}C label.

the ^{13}C-NMR analysis of the formed desmosterol benzoate (**73a** and **73b**, respectively) revealed that the ^{13}C label was distributed almost equally at (*E*)- and (*Z*)-methyl groups. This result suggested a reaction path involving a discrete carbonium ion (at C-25) intermediate.

In addition, the determination of the C-24 stereochemistry of the other reaction products (**74a**/**74b** and **75a**/**75b**), together with the results of an analogous epoxide–carbonyl rearrangement,[55] led to the interesting conclusion that the migration of hydrogen leading to a ketone, e.g., **74**, takes place exclusively with retention of the configuration at the migration terminus, whereas the migration of an alkyl group leading to an aldehyde, e.g., **75**, proceeds exclusively with inversion of configuration at the migration terminus.

Stereochemistry of Desmosterol Hydrogenation. Δ^{24}-Sterol reductase is prevalent in mammals, where it catalyzes the reduction of 24(25)-unsaturated steroid, such as lanosterol and desmosterol.[56] The stereochemistry involved was elucidated in the early 1970s by experiments using radioisotopically labeled compounds.[57,58] Coincidentally, yet notably, a similar reduction is involved in the last step of phytosterol dealkylation. However, information is quite limited on the Δ^{24}-sterol reductase of insect origin.[59] We elucidated the stereochemistry, i.e., direction of the hydrogen addition at the C-25 position, in the reductase action in *B. mori*, again taking advantage of a ^{13}C tracer.

Incubation of isopropylidene (*E*)-methyl ^{13}C-labeled desmosterol[51] with a cell-free preparation from *B. mori* in the presence of NADPH afforded cholesterol labeled at the *pro-R*-methyl group of the isopropyl terminal. The difference in the chemical shifts between *pro-R*-Me (δ_C 22.50) and *pro-S*-Me (δ_C 22.80) was sufficient to assign the methyl groups unambiguously.[60,61] The results imply that the C-25 hydrogen was introduced stereospecifically from the *si* face of the double bond (Figure 7).[62] The observed stereochemical course in *B. mori* is coincident with that found in the transformation of lanosterol into cholesterol in rats.[58]

Since retention of the configuration at C-25 in the preceding two steps, i.e., dehydrogenation of sitosterol and epoxidation of the resultant fucosterol, may reasonably be assumed, the overall behavior of the diastereotopic methyl groups can be depicted as in Figure 1. Included also in Figure 1 is the fate of C-2 of mevalonate during sitosterol biosynthesis in plants.[63] It can be seen that this C-2 is transformed into the *pro-S*-methyl of sitosterol in plants,[63] and eventually into the *pro-S*-methyl of cholesterol in *B. mori*. This is in contrast with the biosynthesis of mammalian cholesterol, where the *pro-R*-methyl is derived from C-2 of mevalonate.[60]

Concluding Remarks

Insects cleverly obtain indispensable sterol by the modification of C-24-alkylated dietary sterols. Since this process is extremely important for insects, they have evolved a diversified enzyme system with an excellent ability to dealkylate a variety of phytosterols, regardless of whether the C-24 alkyl group is methyl or ethyl, whether the stereochemistry at C-24 is *R* or *S*, and whether the C-22 double bond is present or absent. However, it should be noted that some insects, e.g., honeybees, are unable to dealkylate sterols.[64] The dealkylation of phytosterol is carried out by three successive reactions: dehydrogenation, epoxidation, and epoxide fragmentation. The resulting desmosterol is eventually hydrogenated to cholesterol. The crucial step is fragmentation of epoxide, and this reaction appears to occur in a highly stereoselective manner, as evidenced from the *pro-S*- and *pro-R*-methyl groups of fucosterol (24*R*,28*R*)-epoxide being transformed to the (*Z*)- and (*E*)-methyl groups, respectively, of desmosterol. It is of significance that the dealkylation mechanism is not the reverse of alkylation of Δ^{24}-sterol, which is a

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final step of phytosterol biosynthesis.[65] Otherwise, insects would be in competition with plants on which they rear, for a common reaction (alkylation/dealkylation) in the opposite direction. Although the insect strategy of phytosterol utilization is unique, they do not enjoy a monopoly of abundant phytosterols in the plant kingdom. Analogous dealkylation of sterol has been reported to occur in sponges[66] and the protozoa *Tetrahymena pyriformis*,[67] as wll as in the other major class of Arthropoda, the crustaceans.[68]

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Twenty years have passed since the discovery of the boron trifluoride-catalyzed rearrangement of fucosterol epoxide, a biomimetic version of the carbon–carbon cleavage reaction of phytosterol dealkylation in insects. Described in this Account is the fruit of our studies which have been carried out at Tokyo Institute of Technology for this period.

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