The membrane-bound sterol Δ⁵⁺⁻-reductase (24-reductase) catalyzes anaerobic reduction of the 24(25)-enes of lanosterol and other obligatory intermediates of cholesterol biosynthesis from lanosterol. A novel assay method and properties of the 24-reductase are described. More than a 120-fold induction of the 24-reductase activity was achieved by feeding rats a diet containing 5% cholestantryme plus 0.1% lovastatin in chow and by modulating diurnal variation. With this enzyme induction condition, lanosterol was converted efficiently into dihydrolanosterol in both intact hepatic microsomes and freshly isolated hepatocytes only when either miconazole or CO was added to inhibit 14α-demethylation of lanosterol. AR425 cells, which are deficient in 14α-methyl demethylase (14α-DM), exhibit lanosterol 24-reductase activity without addition of either CO or miconazole. Conversely, inhibition of the 24-reductase was not required for the expression of 14α-DM activity. Studies on the substrate specificities for the 24-reductase using different 24(25)-enes showed that the most reactive substrate was 5α-cholesta-7,24-dien-3β-ol, which exhibited a maximal 18-fold higher kcat than that of lanosterol without the aid of the 14α-DM inhibitor. In addition, both the kinetic behaviour of lanosterol substrate in relation to the 24-reductase and a non-competitive inhibition mode of U18666A (Kc, 0.157 μM) as well as Triparanol (Kc, 0.523 μM), two well-known 24-reductase inhibitors, were determined. On the basis of our new findings on the preferred substrate and on the negative effect of 14α-DM on the 24-reductase, we suggest that C-24 reduction of sterols takes place straight after sterol Δ⁵⁺⁻-isomerization of zymosterol, which occurs several steps after C-32 demethylation of lanosterol in the 19-step pathway of cholesterol biosynthesis from lanosterol.

INTRODUCTION

The membrane-bound sterol Δ⁵⁺⁻-reductase (24-reductase) catalyzes anaerobic reduction of the sterol 24(25)-enes, which are obligatory intermediates of cholesterol biosynthesis from lanosterol in mammals (Figure 1) [1–5]. The gene for this enzyme, also known as lanosterol reductase or desmosterol reductase, and those were based on a cumbersome assay protocol that used radioisotope-labelled lanosterol as a substrate [1,2,9]. Since then, virtually no published biochemical studies have attempted to elucidate the substrate specificity of this enzyme, its regulation, or its relationship to other cholesterogenic enzymes [e.g. 14α-methyl demethylase (14α-DM)]. The absence of reports may be due in part to a lack of convenient and rapid assay methods that use unlabelled 24(25)-ene sterol intermediates (e.g. lanosterol, zymosterol and desmosterol) that are commercially available, thus a simple assay has been developed. It is known, however, that in vivo or in vitro inhibition of lanosterol 14α-methyl demethylation by triarimole or azole compounds (e.g. miconazole and ketoconazole) results in large accumulation of dihydrolanosterol, a product of C-24 reduction of lanosterol [10–14]. For example, ketoconazole treatment in humans has resulted in an approx. 248-fold increase in dihydrolanosterol concentration in the serum [14]. These findings strongly suggest that there must be a correlation between 14α-DM inhibition and activation of the 24-reductase, and this correlation could eventually provide a basis for determining the reaction sequence in which lanosterol serves as a common substrate for both enzymes. Because both 14α-DM and 24-reductase act on the same substrate, this represents a branch point in pathways that deserve further investigation.

Here we describe a novel enzyme assay that markedly facilitates determination of the preferred reaction site through substrate specificity as well as an investigation of the regulation of activity of the 24-reductase, and its relationship to 14α-DM which appears to be crucial for the C-24 reduction of lanosterol at a branch point in the pathway.

MATERIALS AND METHODS

Animals and diet
Male Sprague–Dawley rats (200–250 g body weight) were maintained on a standard rodent chow or a chow supplemented with

Abbreviations used: AY-9944, trans-1,4-bis-(2-chlorobenzylaminomethyl)cyclohexane dihydrochloride; CL, 5% cholestantryme plus 0.1% lovastatin (diet); desmosterol, 5α-cholesta-5,24-dien-3β-ol; 14α-DM, 14α-methyl demethylase; lanosterol, 4,4′,14α-trimethyl-5α-cholesta-8,24-dien-3β-ol; lathosterol, 5α-cholesta-7-en-3β-ol; NB-598, (E)-N-ethyl-N-(66-dimethyl-2-hepten-4-ynyl)-3-[[(3,3′-bithiochinen-5-yl)methoxy]benezemethanamine; triarimole, α-(2,4-dichlorophenyl)-α-phenyl-5-pyrimidine methanol; zymosterol, 5α-cholesta-8,24-dien-3β-ol.

1 To whom correspondence should be addressed.
various agents under a reverse light cycle (light 18:00–06:00 h) unless otherwise specified. These diets are essentially as described previously [15–17]: normal chow, 5% cholestanol, 0.1% lovastatin, 5% clofibrate plus 0.1% lovastatin (CL diet), 0.01% \textit{trans}-1,4-bis-(2-chlorobenzylaminomethyl)cyclohexane dihydrochloride (AY-9944) and 0.2% miconazole.

Preparation of microsomes, sterol substrates and enzyme assays

Rats were killed by decapitation at the midpoint of the dark period and their livers were excised and processed for microsome preparation as previously described [17]. Sterol substrates (lanosterol, desmosterol, zymosterol and 5α-cholesta-7,24-dien-3β-ol) were prepared as previously described [18,19]. The 24-reductase was assayed as follows. Lanosterol (or other sterol) (110 nmol) in detergent suspension that had been made by addition of Triton WR-1339 (detergent/sterol, 75:1) [18,19] was added to an assay mixture (total volume 1.0 ml in 0.1 M Tris/HCl buffer, pH 6.2, 1 mM GSH, 10 mM nicotinamide, 0.5 mM EDTA, 20% (w/v) glycerol, 0.3–2 mg of protein, 2 mM NADPH, 20 nmol of miconazole, 10 mM AY-9944, 25 mg of glucose and 20 units of glucose oxidase, designated buffer A) that had been preincubated under nitrogen at 37 °C for 4 min. The addition of AY-9944 was necessary to completely prevent concomitant Δ^4-Δ^2 isomerization of zymosterol or Δ^2-reduction of 5α-cholesta-7,24-dien-3β-ol only when these sterols were used as substrate in the substrate-specificity study. In some experiments, the incubation mixture was degassed with CO for a few minutes before the substrate was added. This CO treatment was routinely performed when the 14α-DM activity had to be blocked because addition of miconazole was omitted. The complete mixture was incubated anaerobically in sealed flasks for 10 min at 37 °C unless otherwise indicated. Incubation was terminated by the addition of 1 ml of ethanolic KOH followed by heating under reflux for 10 min. Sterols were extracted with 4 vol. of petroleum ether and dried under N\textsubscript{2} gas followed by GLC analysis at high-sensitivity attenuation [19]. Enzymic activity was calculated from the relative amounts of diene substrate and monoene product in incubated samples compared with unincubated controls. Throughout this study the only chromatographically detectable endogenous sterol of consequence in microsomal protein was cholesterol. Anaerobic conditions prevented other sterol transformations in the incubation mixture. Assay of 14α-DM was carried out using 1.0 mg of microsomal protein from rats on the CL diet in the presence of 2 mM NADPH, 1 mM KCN and 300 μM lanosterol for 20 min, at which point the initial reaction rate can be observed as described previously [20].

Preparation of rat hepatocytes and enzyme assay

Rat hepatocytes were prepared as previously described [17]. Enzyme assay was performed using total cell extracts (0.5–2 mg) as described above. Protein concentration was determined by the Lowry assay [21] with BSA as standard.

Maintenance of cultured cells

Chinese hamster ovary (CHO-K1) cells and AR45 cells were grown in modified McCoy’s 5a medium (Gibco) supplemented with 2.5% fetal bovine serum as described previously [13]. In some experiments, AR45 cells and CHO cells were grown in McCoy’s 5a medium supplemented with 1%, delipidated fetal bovine serum for 18 h before harvest. Cells were collected by scraping and washed twice with PBS, followed by suspension in buffer A.

Analytical procedures

Microsomal cholesterol and other sterols (lanosterol and dihydrolanosterol) contained in the enzymic reaction mixtures were measured by GLC. GLC was performed with a Young-In 680D or Hewlett-Packard 5890II gas chromatograph using a capillary column (SAC-5; 5% diphenylsiloxane; 30 m × 0.25 mm; 0.25 μm internal diameter). Analyses were carried out at 280 °C with a N\textsubscript{2} carrier gas at a flow rate of 2.44 ml/min. Sterols were quantified relative to a standard of 5α-cholestanole using a flame ionization detector. The relative retention times (compared with a cholesterol standard) were 1.476 for lanosterol (substrate) and 1.362 for dihydrolanosterol (product). The relative retention times of other sterols and compounds (compared with cholesterol) were as follows: zymosterol, 1.115; 5α-cholesta-7,24-dien-3β-ol, 1.162; 5α-cholesta-7-en-3β-ol (lath-
GLC–MS analysis of enzymic reaction products

GLC–MS analysis was conducted on a Hewlett-Packard 5988 GLC–MS system. The GLC conditions were: oven temperature, 250 °C; injection port, 300 °C; Ultra-1 column (25 m × 0.2 mm × 0.33 µm); carrier gas, helium; flow rate, 0.5 ml/min. The MS conditions were: ionization mode, electron ionization and chemical ionization; source temperature, 250 and 200 °C; electronvolts, 70 and 200; ionizing gas, methane. Sterols were dissolved in methylene chloride, and 2 µl aliquots were injected for analysis.

Reagents, drugs and other materials

The sources of the following drugs or agents are indicated in parentheses: AY-9944 (Dr. D. Dvornik, Wyeth–Ayerst, Princeton, NJ, U.S.A.); cholestyramine (LG Chem, Pharmaceutical Division, Seoul, Korea); Triparanol (Dr. H. W. Bohme, Marion Merrell Dow Research Institute, Cincinnati, OH, U.S.A.); U18666A (Dr. R. J. Cenedella, Kirksville College of Osteopathic Medicine, Kirksville, MO, U.S.A.); Lovastatin® (Dr. Y.-K. Sim, Choongwae Pharmaceutical Co., Suwon, Korea) and Squa- lestatin I (Dr. A. Baxter, Glaxo Group Research Ltd., Greenford, Middlesex, U.K.). Miconazole and ketoconazole were obtained from Sigma and Korea Janssen Pharmaceutical Co. (Seoul, Korea). NADP⁺, NADPH and mevalonolactone were obtained from Sigma. Sterols including lanosterol, 25-hydroxycholesterol and cholesterol were from Steraloid or Sigma. Collagenase was obtained from Gibco, and most of the other tissue-culture media and supplements were from Sigma. All other reagents were the best grade available.

RESULTS

Identification of the 24-reductase reaction products

Peaks 1 and 2 in Figure 2(A) correspond to authentic lanosterol (m/z 426 in Figure 2B, i) and 24,25-dihydrolanosterol (m/z 428 in Figure 2B, ii), which are the substrate and product of the 24-reductase respectively. This result reveals that lanosterol can be converted very efficiently into dihydrolanosterol by the 24-reductase in the presence of miconazole.

Regulation of enzyme activity by a combination of CL-diet feeding plus diurnal variation

To determine whether sterol 24-reductase activity can be regulated by feeding cholesterol-lowering drugs, such as lovastatin and cholestyramine, enzyme assay was performed anaerobically using microsomes that had been prepared from rats fed on either the normal diet or the CL diet [16,17], in the presence of miconazole, which specifically inhibits the 14α-methyl demethylation of lanosterol [22,23]. Figure 3 shows a representative time course of lanosterol conversion into dihydrolanosterol (top panel), and the effects of protein amount (middle panel). Interestingly, more than a 40-fold induction of enzyme activity was achieved by feeding rats the CL diet (e.g. for a 30 min incubation, the maximum was 7.40 compared with 0.18 nmol/min per mg) (Figure 3, top). A nearly identical pattern of 24-reductase activity was observed in hepatocytes that had been freshly isolated from rats fed on either the CL diet for 7 days or the normal chow (results not shown). The protein amount and time for the half-maximum reaction velocity for the microsomal proteins from rats on the CL diet, estimated from the curves, were about 10 min and 0.5 mg respectively (Figure 3, top and middle panels). In addition, C-24 reduction was found to be maximal at 2 mM NADPH and at pH 6.5, at which point the activity decreased in both the acid and alkaline directions (results not shown). To examine whether 24-reductase activity is influenced by the circadian rhythm, rats were fed on the normal chow or CL diet for 7 days and killed to determine changes in activity at 2–4 h intervals for a 24 h period from the beginning of the eighth day. Figure 3 (bottom) shows that only the microsomal 24-reductase activity obtained from the CL-diet-fed rats exhibits a striking diurnal variation, with an approx. 3-fold increase in enzyme activity at 22:00 h. Considering diet-mediated induction plus diurnal variation together, more than a 120-fold enzyme induction was actually achieved relative to the control (i.e. to the activity of the microsomes prepared from rats fed normal chow during the light cycle period).
Relationship between 14α-DM and lanosterol 24-reductase

To examine whether there is a direct relationship (i.e. competition for the substrate) between 14α-DM and the 24-reductase, both of which use lanosterol as substrate, the effects of 14α-DM inhibitors were assessed. Figure 4 (top) shows dose-dependent stimulation of the 24-reductase activity by miconazole or ketoconazole. There was almost no reaction product in the absence of miconazole or ketoconazole under normal conditions. Maximal activity was observed when little more than 10 µM miconazole was added to the reaction mixture. Ketoconazole, which is also a known 14α-DM inhibitor [22–24], appears to be less effective. Because addition of AY-9944 to the miconazole-treated samples did not change the reaction rate significantly (Figure 4, top), 10 µM miconazole was used throughout the experiments.

To examine whether this phenomenon could be observed in the presence of CO, another potent 14α-DM inhibitor, we treated the incubation mixture with CO for various lengths of time before adding the substrate, and incubated it for 5 min. Figure 4 (bottom) illustrates that the 24-reductase activity was stimulated in a time-dependent manner when 14α-DM activity was blocked by CO treatment, which would not be expected to displace substrate from the demethylating enzyme. Meanwhile, 14α-DM activity was not affected by the presence of 100 µM Triparanol, a specific inhibitor of 24-reductase (results not shown). Thus this result indicates that blockade of 14α-DM is a prerequisite for the detection of 24-reductase activity when lanosterol is used as substrate. Conversely, inhibition of 24-reductase is not required at all for the detection of 14α-DM activity (results not shown).

Expression of the 24-reductase activity in cells that lack 14α-DM (AR45 cells)

To corroborate this relationship between the two enzymes, 24-reductase activity was assessed in AR45 cells, which lack 14α-DM activity [13]. Figure 5 shows that, in the absence of CO or miconazole, 24-reductase activity with lanosterol is actively expressed in AR45 cells and increases linearly in direct proportion to increasing amounts of cell protein. In contrast, very low activity was detected in CHO cell extracts only when miconazole or CO was present (results not shown), but no activity was detected in the absence of this treatment (Figure 5). Taken together, these results suggest that 14α-DM activity is dominant over 24-reductase activity, and blockade or removal of 14α-DM activity is absolutely required for the detection of maximal 24-reductase activity when lanosterol substrate is present.

Kinetic behaviour and in vitro modulation of 24-reductase activity

Since there have been no reports so far on the kinetic behaviour of the 24-reductase, enzyme assays were carried out to determine apparent $K_m$ and $V_{max}$ under the conditions described above. From the data presented in Figure 6(A), the apparent $K_m$ and $V_{max}$ values for lanosterol 24-reductase were estimated to be about 109 µM and 0.361 nmol/min per mg of protein respectively. Although this enzyme has long been known to be inhibited by the well-known cholesterol-lowering agents Triparanol and U18666A [2,25–27], their inhibitory concentrations and mechanism of action against the 24-reductase have not been reported. To examine how various cholesterol-lowering agents, including Triparanol and U18666A, affect 24-reductase activity in vitro, and to compare their relative potencies, if any, microsomes obtained from rats fed on the CL diet were incubated in the presence of these agents, and the agents were tested for their

Figure 3 Induction of the 24-reductase activity by CL-diet feeding

Top, using 2 mg of protein, the duration of incubation was varied. Middle, using a 10 min incubation, the amount of microsomal protein was varied. In the inset, relative enzyme activities of each diet-fed group at 30 min (top) and at 0.5 mg (middle) are shown for comparison. The letters N and CL represent normal chow diet and 5% cholesteryamine plus 0.1% lovastatin in
Figure 4 Effects of 14α-DM inhibition on the expression of the 24-reductase activity

Top, dose–response curve for the 24-reductase activity in the presence of miconazole, ketoconazole and AY-9944. Enzymic reactions were carried out in the presence of various concentrations of miconazole (E), miconazole plus AY-9944 (F), ketoconazole (D) and AY-9944 (G). Bottom, effect of CO treatment on the expression of the 24-reductase activity. Each sample was pretreated with CO for a defined time before addition of substrate, followed by incubation for 15 min. Each assay was carried out in the absence of miconazole. Data are presented as means ± S.D. for three different determinations performed in triplicate.

Figure 5 Expression of the 24-reductase activity in AR45 cells and CHO cells

The amount of total protein extracts was varied using a 20 min incubation in the absence of miconazole. Each value is the mean of duplicate assays.

Substrate specificity and the preferred reaction sequence for the 24-reductase

As illustrated in Figure 1, the 24-reductase catalyses many reactions, but their relative sequence has not been established so far because of lack of definitive studies on the substrate specificity of the enzyme. This may be due to the lack of a convenient assay system for the enzyme. To gain information on the sequence of reactions in which the 24-reductase is involved, we first selected representative potential sterol intermediates containing the 24,25-double bond and examined the substrate preference for this enzyme in the presence or absence of miconazole or CO. We based the selection of the 24(25)-ene sterol intermediates on work reported from other laboratories [5,8,12,21,29] that suggests that C-24 reduction occurs either before or after, but not during, C-30, C-31 and C-32 demethylation of lanosterol. We therefore prepared only essential representative sterol intermediates: lanosterol, zymosterol, 5α-cholesta-7,24-dien-3β-ol and desmosterol. The purpose of this experiment was twofold: (1) determination of the most reactive (preferred) sterol substrate for the 24-
reductase; (2) examination of the requirement for miconazole or CO treatment for detection of the 24-reductase activity when a 24(25)-ene sterol intermediate other than lanosterol is used. The data summarized in Table 1 demonstrate that the preferred substrate for the 24-reductase is 5α-cholesta-7,24-dien-3β-ol, an enzymic product of the sterol 8-isomerase reaction [16,19], and this is followed by zymosterol, desmosterol and lanosterol. The reaction rate with 5α-cholesta-7,24-dien-3β-ol is consistently higher than with the other sterols regardless of the presence of either CO or miconazole in the enzyme assay reaction. Furthermore, as shown in Table 2, $k_{cat}$ of 7,24-diene is about 18-fold higher than that of lanosterol, indicating that this sterol is the preferred substrate for the 24-reductase. Taken together, these results suggest that the 24-reduction of sterol must occur just after the sterol $\Delta^{24}$ isomerization, which of course does not require the presence of 14α-DM inhibitor or CO, which is absolutely required when lanosterol is used as substrate.

**DISCUSSION**

The major objectives of the present studies were fourfold: (1) development of novel assay procedures by which the 24-reductase activity can be easily detected; (2) determination of the mode of regulation of the 24-reductase by cholesterol-lowering agents and diurnal variation; (3) determination of the relationship between the lanosterol-utilizing enzymes, 14α-DM and the 24-reductase; (4) determination of the previously unknown reaction sequence of sterol 24-reduction.
First, our novel assay system for the 24-reductase employing the GLC system has been found to be much easier, more convenient, safer and faster than the one previously reported by Avigan et al. [1], which mainly depended on non-available radiolabelled substrates. With this assay developed for optimal conditions, there is no longer a requirement for any other subcellular components, such as S_{sub} fractions, and long incubation times [1,2].

Secondly, like other cholesterogenic enzymes, the 24-reductase activity can be highly regulated by either modulation of the circadian rhythm or feeding cholesterol-lowering agents such as cholestyramine and lovastatin in the diets (Figure 3). The CL diet is a commonly used diet for inducing most cholesterogenic enzymes, such as hydroxymethylglutaryl-CoA reductase [15], squalene synthase [30], squalene epoxidase [29], sterol 14-reductase [17] and sterol 8-isomerase [16]. However, a more than 120-fold induction of 24-reductase activity by the CL diet plus modulation of diurnal variation is the largest observed for any enzyme involved in the pathway between lanosterol and cholesterol [16–19]. These induction effects of diet on the 24-reductase in vivo usually reached a peak after 7 days of feeding and then gradually declined (results not shown). At this time point, feeding cholestyramine (5%), lovastatin (0.1%), miconazole (0.2%) or AY-9944 (0.01%) alone resulted in about 20–60% of the induction mediated by the CL diet (results not shown). Thus the CL diet was essential for maximal lanosterol 24-reductase activity in the presence of miconazole (see below).

Thirdly, we wanted to determine how the blockade of 14α-DM, an enzyme that also uses lanosterol as substrate, would affect the 24-reductase activity in order to clarify the relationship between these two enzymes. Surprisingly, in the presence of miconazole or ketoconazole, the 24-reductase activity was actually stimulated in a dose-dependent manner (Figure 4, top), suggesting a negative effect of 14α-DM on 24-reductase. Results on substrate specificity summarized in Tables 1 and 2 led to the conclusion that lanosterol is not normally subjected to C-24 reduction unless cytochrome P-450 14α-DM is inhibited or deficient (AR45 cells). Furthermore, in the absence of miconazole or CO, 24(25)-ene sterols other than lanosterol have been shown to be efficiently converted into 24(25)-dihydrosterols, indicating that 14α-DM did not have to be inhibited for the 24-reductase activity for these non-lanosterol 24(25)-ene substrates to be observed (Tables 1 and 2). The inhibition studies also indicate that the lanosterol substrate for 24-reductase may be bound by 14α-DM. Considering the reported IC_{50} values for miconazole (0.9 μM) and ketoconazole (0.4 μM) [31], their stimulation of the 24-reductase activity by restraining 14α-DM activity appears to result in the reverse of what might be expected. Miconazole is a competitive inhibitor of rat liver microsomal lanosterol 14α-DM [12,13,23] and human placental aromatase [24]. The inhibition of aromatase as well as 14α-DM appears to be due to the direct interaction of miconazole with cytochrome P-450 [22]. Earlier reports indicated that, when 14α-DM activity is strongly inhibited by miconazole in vitro, there is a huge accumulation of dihydrolanosterol [12,13]. This suggests that the 24-reductase, which is normally in competition with 14α-DM, catalyses C-24 reduction in the presence of miconazole. The present data prove that the cause of this azole-mediated accumulation of dihydrolanosterol was actually the activation of 24-reductase by the inhibition of 14α-DM activity.

### Table 1: Effect of 14α-DM inhibition on 24-reductase activity with different sterol substrates

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Miconazole...</th>
<th>CO...</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lanosterol (4,4′,14α-trimethyl-5α-cholesta-8,24-dien-3β-ol)</td>
<td>N.D.</td>
<td>0.277 ± 0.003 (100)</td>
</tr>
<tr>
<td>Zymosterol (5α-cholesta-8,24-dien-3β-ol)</td>
<td>0.911 ± 0.004</td>
<td>0.945 ± 0.002 (341)</td>
</tr>
<tr>
<td>5α-Cholesterol-7,24-dien-3β-ol</td>
<td>1.120 ± 0.006</td>
<td>1.121 ± 0.004 (476)</td>
</tr>
<tr>
<td>Desmosterol (5α-cholesta-5,24-dien-3β-ol)</td>
<td>0.745 ± 0.003</td>
<td>0.831 ± 0.005 (300)</td>
</tr>
</tbody>
</table>

**Table 2: Substrate specificity of the 24-reductase**

Enzyme assays were carried out using 0.5 mg of microsomal protein under anaerobic conditions for 10 min at 37°C in the presence or absence of miconazole and CO as described in the Materials and methods section. Each value represents the mean ± S.D. obtained from three separate experiments, each experiment being carried out in triplicate. The inhibition studies also indicated that the lanosterol substrate for 24-reductase may be bound by 14α-DM, N.D., Not detected.

<table>
<thead>
<tr>
<th>Substrate sterol</th>
<th>V_{max} (nmol/min per mg of protein)</th>
<th>K_{m} (μM)</th>
<th>k_{cat} (V_{max}/K_{m})</th>
<th>Relative k_{cat} (fold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lanosterol (4,4′,14α-trimethyl-5α-cholesta-8,24-dien-3β-ol)</td>
<td>0.361</td>
<td>109</td>
<td>0.0033</td>
<td>1.0</td>
</tr>
<tr>
<td>Zymosterol (5α-cholesta-8,24-dien-3β-ol)</td>
<td>3.550</td>
<td>176</td>
<td>0.0201</td>
<td>6.1</td>
</tr>
<tr>
<td>5α-Cholesterol-7,24-dien-3β-ol</td>
<td>2.176</td>
<td>37</td>
<td>0.0586</td>
<td>17.8</td>
</tr>
<tr>
<td>Desmosterol (5α-cholesta-5,24-dien-3β-ol)</td>
<td>2.945</td>
<td>163</td>
<td>0.0180</td>
<td>5.5</td>
</tr>
</tbody>
</table>

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Finally, data outlined in Tables 1 and 2 strongly suggest that C-24 reduction of sterol intermediates takes place straight after the sterol Δ7-9 isomerization in the 19-step conversion of lanosterol into cholesterol. Conversely, under normal conditions, the first reaction in the 19-step lanosterol transformation into cholesterol should be catalysed by the 14α-DM, and this has been considered to be the rate-limiting step [8]. On the basis of this finding and previous reports from other laboratories [5,8,12,22,31], we propose that the logical sequence of lanosterol transformation to cholesterol in the normal physiological state (i.e. under conditions in which there is no interruption of cytochrome P-450 14α-DM activity) would be: lanosterol C-32 demethylation → C-30 plus C-31 demethylation → Δ7,24-isomerization of zymosterol → C-24 reduction of Δ7,24-diene → Δ7-dehydrogenation of lathosterol → Δ9 reduction of 7-dehydrocholesterol → cholesterol. Data that could strengthen this proposal will be available when the purified individual enzymes of the complex pathway from lanosterol to cholesterol can be reconstituted.

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