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Stereochemistry of reduction of the C-24,25 double bond in the conversion of desmosterol into cholesterol

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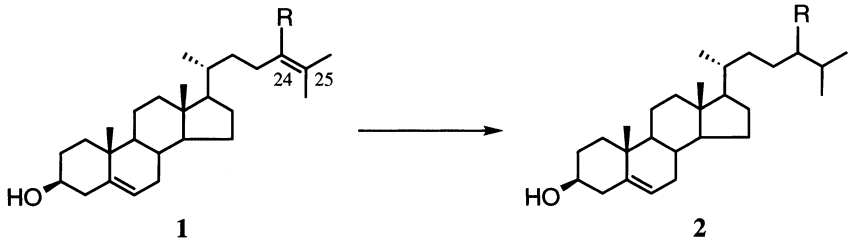
Abstract—Feeding of the chemically prepared [24-^{13}C, 24-^{2}H]desmosterol to cell-free systems derived from rat liver and silkworm gut and to cultured cells of *Oryza sativa* followed by deuterium-decoupled ^{1}H, ^{13}C shift correlation NMR analysis of the biosynthesized cholesterol revealed the stereospecific incorporation of hydrogen atoms from the *re*-face of the C-24 position of desmosterol.

The final stage of sterol biosynthesis involves reduction of Δ^{24} olefinic sterols such as desmosterol (**1**, R = H), 24-methyldesmosterol (**1**, R = Me) and 24-ethyldesmosterol (**1**, R = Et), which were established as the biosynthetic precursors of cholesterol (**2**, R = H), campesterol/dihydrobrassicasterol (**2**, R = Me) and sitosterol (**2**, R = Et), respectively (Scheme 1).

In regard to stereochemistry of the biohydrogenation reaction catalyzed by rat liver 3β-hydroxysterol Δ^{24}-reductase (Δ^{24}-sterol reductase) producing cholesterol, a *syn* addition of hydrogen atoms on the 24-*si* face and 25-*si* face of the C-24,25 double bond was proposed,[1] whereas an *anti* addition was reported for tigogenin (and thence cholesterol) biosynthesis in *Digitalis lanata*.[2] We have recently demonstrated with cultured cells of *Oryza sativa* that 24-methyldesmosterol and 24-ethyldesmosterol are converted into campesterol/dihydrobrassicasterol and sitosterol, respectively, by an *anti* addition of hydrogen atoms on the respective Δ^{24} olefinic precursors.[3] In this paper we have reexamined the stereochemical course of the reduction of desmosterol (1, R = H) leading to cholesterol (**2**, R = H) in rat liver, as well as in insects and higher plants. The results clearly demonstrated that an *anti* addition uniformly occurs, irrespective of the species examined, in contradiction to the dogma which has prevailed for 30 years.[1]

Because stereospecific hydrogen introduction on C-25 from the *si*-face during cholesterol biosynthesis was amply demonstrated in various species,[1,4] we have presently focused on the stereochemical problem at C-24, namely, whether the newly introduced hydrogen on C-24 occupies the pro-*R* or the pro-*S* position in cholesterol. This problem is difficult to solve, since C-24 of cholesterol is a prochiral carbon, which is located



Scheme 1. Reduction of Δ^{24}-sterols (**1**) to yield naturally abundant sterols (**2**).

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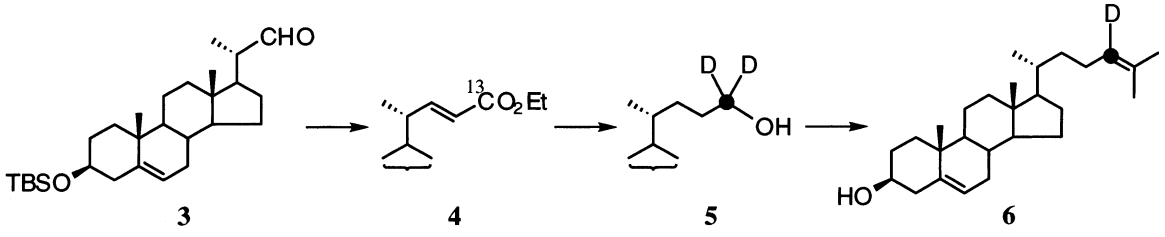
four carbons apart from the nearest chiral carbon (C-20). The strategy of feeding of [24-^{13}C,24-^{2}H]desmosterol (**6**) followed by deuterium-decoupled ^{1}H, ^{13}C shift correlation NMR analysis[5] of the biosynthesized cholesterol appears promising, as evidenced from our recent investigation on stereochemical details occurring at the C-28 methylene group during sitosterol biosynthesis.[6]

The required substrate [24-^{13}C, 24-^{2}H]desmosterol (**6**) was prepared as shown in Scheme 2.[7] Horner–Emmons reaction of the 3-*t*-butyldimethylsilyl(TBS)-oxy-22-al (**3**) derived from bisnorcholenic acid with [1-^{13}C]triethyl phosphonoacetate (Aldrich) gave the α,β-unsaturated ester **4** which on catalytic hydrogenation with PtO_2 in ethanol followed by reduction with lithium aluminum deuteride afforded [24-^{13}C,24-$^{2}H_2$]-24-alcohol (**5**). Oxidation of **5** with tetra-*n*-propylammonium perruthenate/*N*-methylmorpholine *N*-oxide, Wittig olefination and desilylation produced [24-^{13}C, 24-^{2}H]desmosterol (**6**) in good yield. Incubation of this substrate **6** with the 24,000×*g* supernatant fraction of rat liver homogenate and the 1500×*g* supernatant fraction prepared from the guts of silkworm larvae (*Bombyx mori*), and with the cultured cells of *O. sativa* was carried out as described previously.[4] Sterol fractions were separated by silica gel chromatography and cholesterols were further purified by HPLC. The cholesterol samples were analyzed with deuterium-decoupled ^{1}H, ^{13}C shift correlation NMR (Fig. 1). The approximate conversion yield of cholesterol from desmosterol was estimated to be 80% in rat liver, 50% in *B. mori* and 0.9% (based on desmosterol added to the culture medium) in *O. sativa*.



Scheme 2. Synthesis of [24-^{13}C, 24-^{2}H]desmosterol (**6**).

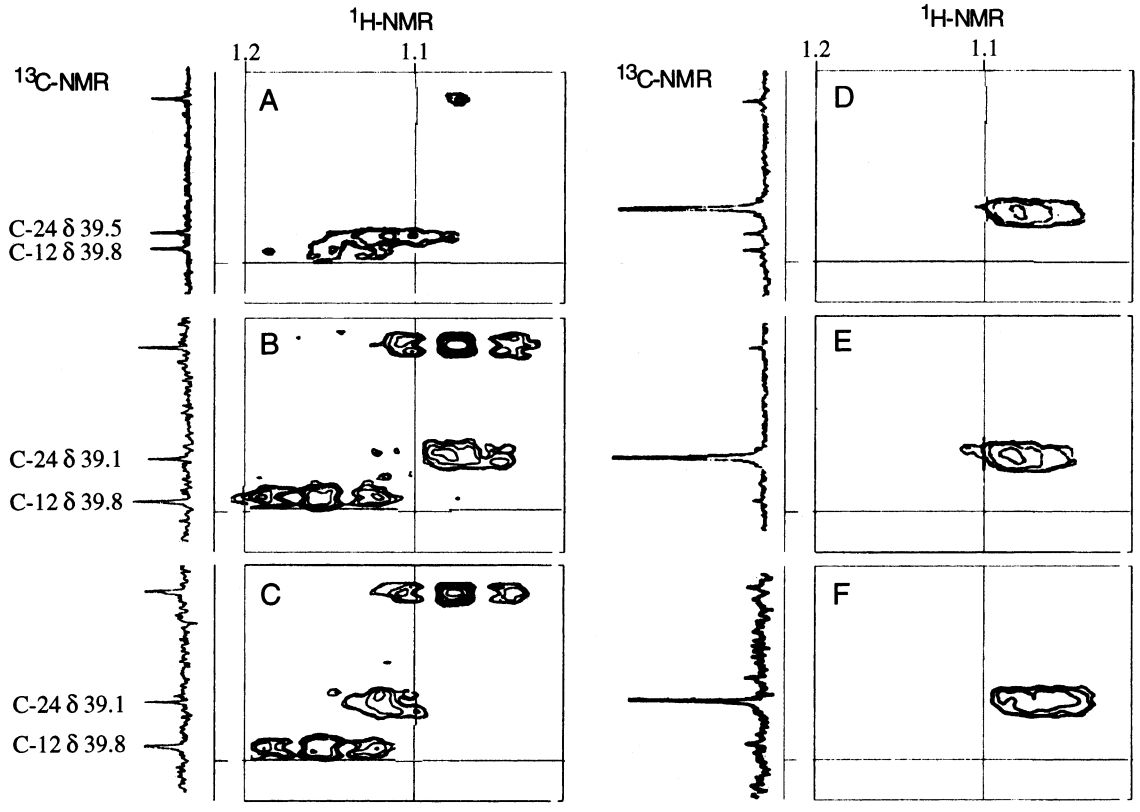
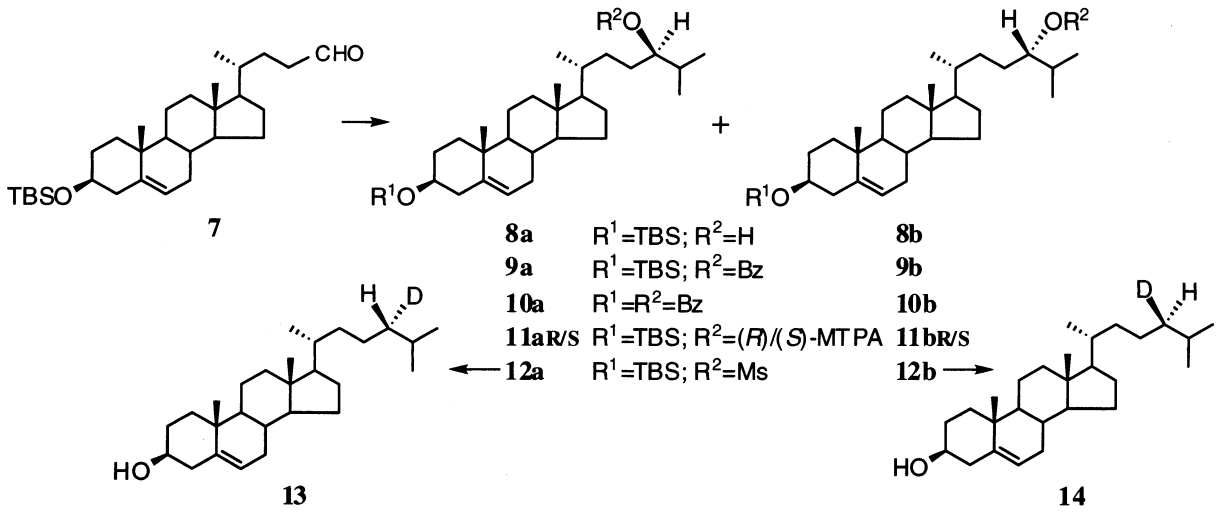
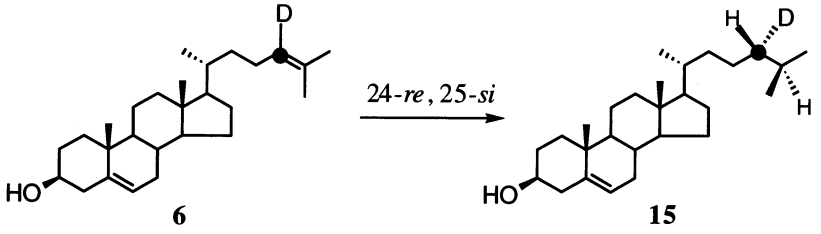


Figure 1. Deuterium-decoupled HMQC spectra of cholesterol (500 MHz for ^{1}H/125 MHz for ^{13}C, in $CDCl_3$). (A) Non-labeled cholesterol; (B) (24*S*)-[24-^{2}H]cholesterol (**13**); (C) (24*R*)-[24-^{2}H] cholesterol (**14**); (D–F) biosynthesized cholesterol from [24-^{13}C, 24-^{2}H]desmosterol (**6**) on incubation with rat liver homogenate (D), with cultured cells of *O. sativa* (E) and with silkworm gut homogenate (F).



Scheme 3. Synthesis of (24*S*)-[24-^{2}H]cholesterol (**13**) and (24*R*)-[24-^{2}H]cholesterol (**14**).



Scheme 4. Conversion of [24-^{13}C, 24-^{2}H]desmosterol (**6**) into (24*S*)-[24-^{13}C, 24-^{2}H]cholesterol (**15**).

In order to aid unequivocal assignment of the chemical shifts of the pro-*R* and pro-*S* hydrogens at C-24 of cholesterol, stereochemically defined [24-pro-*S*-^{2}H] and [24-pro-*R*-^{2}H] cholesterols (**13** and **14**), were synthesized (Scheme 3). Grignard reaction of the 3-OTBS-24-al (**7**) derived from cholenic acid gave a diastereomeric mixture of (24*R*)- and (24*S*)-24-alcohol, (**8a**) and (**8b**), whose benzoates (**9a** and **9b**) were prepared and separated by HPLC (TOSOH TSK-GEL, SILICA-60, 7.8× 300 mm, *n*-hexane-dichloromethane 3:1, 6.5 ml/min). The less mobile benzoate (**9a**) and the more mobile isomer (**9b**) were derivatized to 3-OTBS-24-ol (**8a** and **8b**), 3,24-dibenzoates(**10a** and **10b**), (*R*)- and (*S*)-2-methoxy-2-phenyl-2-(trifluoromethyl)acetic acid (MTPA) esters (**11aR/S** and **11bR/S**), and methanesulfonates (**12a** and **12b**). The configurations at the C-24 position of these compounds were determined by ^{13}C NMR of 3-OTBS-24-ol (**8**) and 3,24-dibenzoates(**10**) compared with our previously reported ones,[8] and further confirmed by application of the advanced Mosher's method[9] for MTPA esters (**11**).[10] Reductive substitution of (24*R*)- and (24*S*)-mesylate (**12a** and **12b**) with sodium borodeuteride in HMPA followed by desilylation afforded (24*S*)-[24-^{2}H]cholesterol (**13**) and (24*R*)-[24-^{2}H]cholesterol (**14**), respectively.

The {^{1}H}{^{2}H}HMQC spectra of **13** and **14** are included in Figure 1. It can be seen that non-labeled cholesterol showed a broad cross-peak at δ 39.5 (24-C)/δ 1.15–1.08 (24-H) (Fig. 1 A), whereas the corresponding peak of 24-deuterated samples (**13**) and (**14**) appeared at δ 39.1 (24-C)/δ 1.08 (24-H) (Fig. 1 B) and δ 39.1 (24-C)/δ 1.12 (24-H) (Fig. 1 C), respectively. These shifted values are consistent with the expected α-isotope shift (^{13}C NMR) and β-isotope shift (^{1}H NMR).[5] Thus, Figure 1 A, B and C allowed definitive stereochemical assignment that the pro-*R* and pro-*S* hydrogens at C-24 appear upfield and downfield, respectively. Figure 1 D, E and F show spectra of the cholesterol biosynthesized in rat liver, *O. sativa* and silkworm gut, respectively. Almost single and intense peaks were observed at δ 1.08, indicating that these peaks are due to the pro-*R* hydrogen at C-24. It is concluded that the biohydrogenation of [24-^{13}C,24-^{2}H]desmosterol (**6**) furnishes (24*S*)-[24-^{13}C,24-^{2}H]cholesterol (**15**) (Scheme 4), implying that introduction of hydrogen into C-24 of desmosterol occurs from the *re*-face.[11] Since 25-*si* face addition was well established,[1,4] the present work demonstrates that addition of hydrogen on the 24(25)-double bond of desmosterol takes place in an *anti* fashion from the *re*-face of C-24 and the *si*-face of C-25, as opposed to a *syn* addition proposed by Caspi and co-workers 30 years ago.[1]

References

1. (a) Greig, J. B.; Varma, K. R.; Caspi, E. *J. Am. Chem. Soc.* **1971**, *93*, 760–766; (b) Kienle, M. G.; Varma, R. K.; Mulheirn, L. J.; Yagen, B.; Caspi, E. *J. Am. Chem. Soc.* **1973**, *95*, 1996–2001; (c) Yagen, B.; O'Grodnick, J. S.; Caspi, E. *J. Chem. Soc., Perkin Trans. 1* **1974**, 1994–2000.

2. (a) Canonica, L.; Ronchetti, F.; Russo, G. *J. Chem. Soc., Perkin Trans. 1* **1974**, 1670–1674; (b) Ronchetti, F.; Russo, G. *J. Chem. Soc., Perkin Trans. 1* **1976**, 1823–1825.
3. (a) Fujimoto, Y.; Sato, N.; Iwai, K.; Hamada, H.; Yamada, J.; Morisaki, M. *Chem. Commun.* **1997**, 681–682; (b) Fujimoto, Y.; Sato, N.; Okuzumi, T.; Yamada, J.; Morisaki, M. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 205–208.
4. (a) Fujimoto, Y.; Nagakari, M.; Ikuina, Y.; Ikekawa, N.; Kakinuma, K. *J. Chem. Soc., Chem. Commun.* **1991**, 688–689; (b) Yagi, T.; Kobayashi, N.; Morisaki, M.; Hara, N.; Fujimoto, Y. *Chem. Pharm. Bull.* **1994**, *42*, 680–682; (c) Nagano, M.; Yamada, J.; Yazaki-Yagi, T.; Morisaki, M.; Nakagawa, T.; Fujimoto, Y. *Chem. Pharm. Bull.* **1997**, *45*, 944–946. Recent progress on 3β-hydroxysterol Δ^{24}-reductase involves the identification of *Arabidopsis DIMINUTO/DWARF1* and human *DHCR24* genes which encode proteins catalyzing the reduction and/or isomerization of $\Delta^{24(28)}$-sterols to $\Delta^{24(25)}$-sterols. Further, it has been reported that DHCR24 protein expressed in *Saccharomyces cerevisiae* belongs to a family of FAD-dependent oxidoreductases (Waterham, H. R.; Koster, J.; Romejin, G. J.; Hennekam, R. C. M.; Vreken, P.; Andersson, H. C.; FitzPatrick, D. R.; Kelly, R. I.; Wanders, R. J. A. *Am. J. Hum. Genet.* **2001**, *69*, 685–694; Klahre, U.; Noguchi, T.; Fujioka, S.; Takatsuto, S.; Yokota, T.; Yoshida, S.; Chua, N. H. *Plant Cell* **1998**, *10*, 1677–1690). However, none of these studies described purification of the reductase, although the enzyme activity was found in a microsomal fraction. Concerning the mechanism of the reduction, it was reported to occur with the addition of a hydrogen from the medium at C-24 and another from NADPH at C-25 (Watkinson, I. A.; Wilton, D. C.; Rahimtula, A. D.; Akhtar, M. M. *Eur. J. Biochem.* **1971**, *23*, 1–6).
5. Reese, P. B.; Trimble, L. A.; Vederas, J. C. *Can. J. Chem.* **1986**, *64*, 1427–1434.
6. Okuzumi, T.; Hara, N.; Fujimoto, Y. *Tetrahedron Lett.* **1999**, *40*, 8863–8866.
7. Corey, E. J.; Grogan, M. J. *Tetrahedron Lett.* **1998**, *39*, 9351–9354.
8. (a) Koizumi, N.; Morisaki, M.; Ikekawa, N. *Tetrahedron Lett.* **1975**, 2203–2206; (b) Koizumi, N.; Fujimoto, Y.; Takeshita, T.; Ikekawa, N. *Chem. Pharm. Bull.* **1979**, *27*, 38–42; (c) Fujimoto, Y.; Ikuina, Y.; Nagakari, M.; Kakinuma, K.; Ikekawa, N. *J. Chem. Soc., Perkin Trans. 1* **1990**, 2041–2046.
9. Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092–4096.
10. Pertinent ^{1}H NMR data for **11aR/S** δ: 0.642/0.664 (18-H_3), 0.835/0.899 (21-H_3), 0.910/0.851, 0.905/0.835 (26-H_3, 27-H_3). For **11bR/S** δ: 0.662/0.638 (18-H_3), 0.919/0.874 (21-H_3), 0.854/0.916, 0.832/0.903 (26-H_3, 27-H_3).
11. Seo and co-workers noted the stereospecific hydrogen attack at C-24 during cholesterol biosynthesis from [2-^{13}C,2-$^2H_3$]acetate in isolated dog hepatocytes, leaving the orientation of the newly introduced hydrogen undetermined: Seo, S.; Saito, H.; Uomori, A.; Yoshimura, Y.; Tonda, K.; Nishibe, Y.; Hirata, M.; Takeuchi, Y.; Takeda, K.; Noguchi, H.; Ebizuka, Y.; Sankawa, U.; Seto, H. *J. Chem. Soc., Perkin Trans. 1* **1991**, 2065–2072.