STEREOCHEMISTRY OF HYDROGEN INTRODUCTION AT C-25 DURING CHOLESTEROL BIOSYNTHESIS IN HIGHER PLANTS

Masa NAGANO,[a] Junko YAMADA,[a] Tomoko YAZAKI-YAGI,[a] Masuo MORISAKI*,[a] Takayoshi NAKAGAWA,[b] and Yoshinori FUJIMOTO*,[b]

Kyoritsu College of Pharmacy,[a] Shibakoen, Minato-ku, Tokyo 105 and Department of Chemistry, Tokyo Institute of Technology,[b] O-okayama, Meguro-ku, Tokyo 152, Japan

Feeding of [26-^{13}C]desmosterol and [26-^{13}C]$\Delta^{25(26)}$-cholesterol to cultured cells of *Oryza sativa* and hairy roots of *Ajuga reptans* var. *atropurpurea* followed by ^{13}C-NMR analysis of the resulting ^{13}C-labeled cholesterol demonstrated that hydrogen introduction at C-25 occurs on the *Si*-face of desmosterol and on the *Re*-face of $\Delta^{25(26)}$-cholesterol.

KEY WORDS desmosterol; Δ^{25}-cholesterol; cholesterol biosynthesis; *Oryza sativa*; *Ajuga reptans* var. *atropurpurea*

The final step in the biosynthesis of cholesterol (**1**) involves the reduction of a Δ^{24}-sterol, *e.g.*, desmosterol (**2**).[1] The stereochemistry of the hydrogen addition at C-25 in this process has been elucidated in rat liver[2,3] and insects,[4] to reveal the 25-*Si*-face attack on desmosterol. The reduction of Δ^{24}-sterols should also occur in plants during the biosynthesis of cholesterol, which is generally a minor sterol in plants, as well as of the steroidal sapogenins, *e.g.*, diosgenin, tigogenin and tokorogenin.[5] This paper reports the stereochemistry of hydrogen introduction at C-25 in *Oryza sativa* and *Ajuga reptans* var. *atropurpurea*, of desmosterol (**2**), and also of $\Delta^{25(26)}$-cholesterol (**3**) which would be an alternative substrate for the C-25 reduction leading to cholesterol. The methodology used is essentially the same as in previous reports.[3,4] Thus, it is determined by ^{13}C-NMR analysis whether [26-^{13}C]cholesterol (**1a**) or [27-^{13}C]cholesterol (**1b**) is produced from the fed substrates of [26-^{13}C]desmosterol (**2**),[6] and [26-^{13}C]$\Delta^{25(26)}$-cholesterol (**3**).[7] (Chart 1)

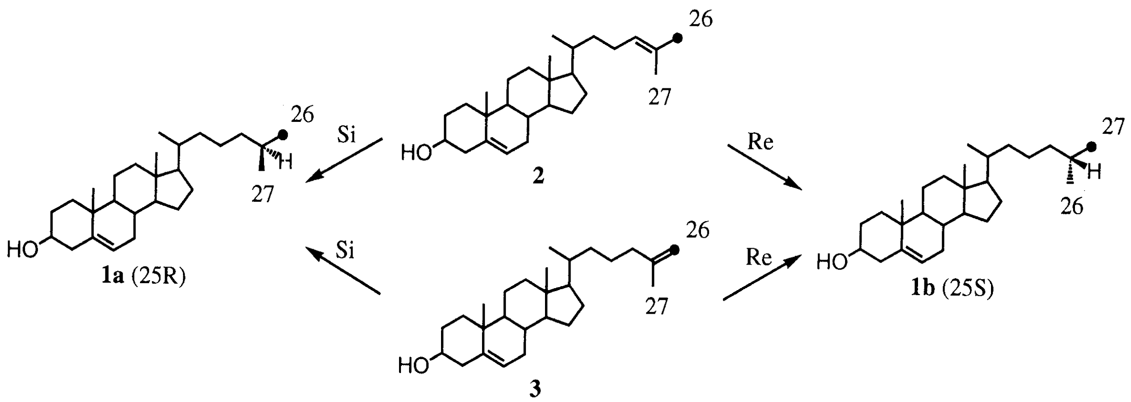

Chart 1

* To whom correspondence should be addressed.

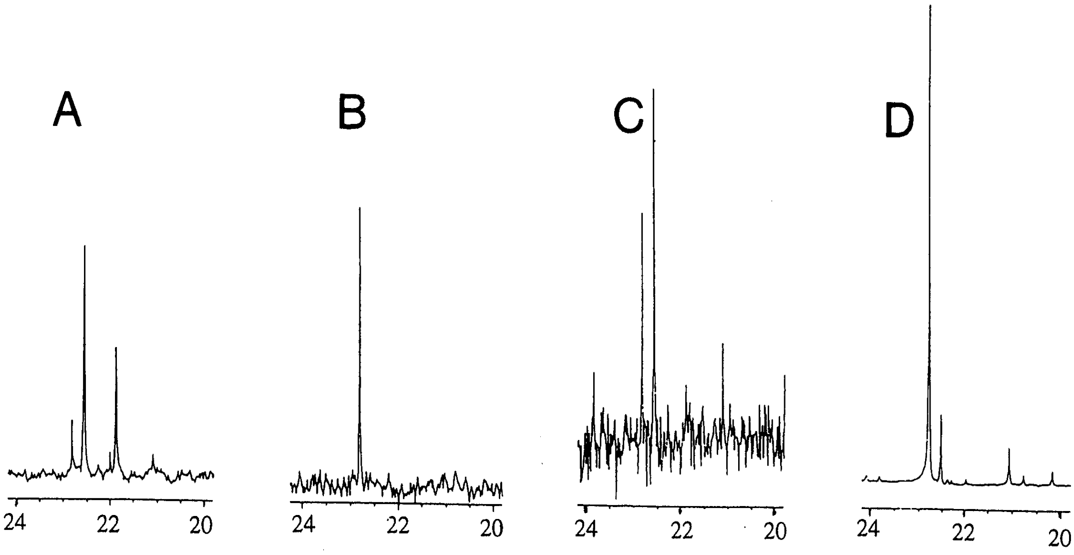

Fig. 1. Partial ^{13}C-NMR Spectra of Cholesterol Biosynthesized from [26-^{13}C] Desmosterol (**A** and **C**) or from [26-^{13}C]$\Delta^{25(26)}$-Cholesterol (**B** and **D**) in *Oryza sativa* (**A** and **B**) or in *Ajuga reptans* var. *atropurpurea* (**C** and **D**)

[26-^{13}C]Desmosterol (**2**, 50.6 mg) was fed to the tissue cultures of *O. sativa* as previously described,[8] and the resulting sterols (22.1 mg) were separated by HPLC to obtain the fraction (1.8 mg) containing cholesterol and 24-methylenecholesterol. ^{13}C-NMR spectra (Fig. 1A) indicated that the *pro-R*-methyl (C-26) of cholesterol appearing at δ 22.5 ppm[9] was labelled with ^{13}C, and therefore came from (*E*)-^{13}C-methyl of desmosterol. In contrast, the intensity of the signal at 22.7 ppm due to *pro-S*-methyl (C-27) was much lower, almost at the same level of other carbons of the endogenous cholesterol. These results imply that cholesterol was produced by the stereoselective 25-*Si*-face addition of hydrogen on desmosterol, in accordance with observations in the rat liver[2,3] and in insects.[4] The ^{13}C-enriched signals at 21.8 ppm in Fig. 1A are due to the *pro-S*-methyl of 24-methylenecholesterol,[10] indicating the transformation of desmosterol (**2**) into this sterol.[11] A feeding experiment of [26-^{13}C]$\Delta^{25(26)}$-cholesterol (**3**) was carried out in the same manner. It is evident from Fig. 1B that the *pro-S*-methyl (C-27) of cholesterol was selectively labeled, implying the 25-*Re*-face addition of hydrogen on $\Delta^{25(26)}$-cholesterol during its transformation into cholesterol. An opposite (25-*Si*-face) attack of hydrogen was reported[12] to occurr in the biosynthesis of 24β-ethylcholesta-7,22-dien-3β-ol (chondrillasterol) from the Δ^{25}-olefinic precursor.

Analogous experiments were carried out by using the hairy roots of *Ajuga*, using the previously reported procedures.[13] When [26-^{13}C]$\Delta^{25(26)}$-cholestrol (**3**) was fed to *Ajuga*, the resulting cholesterol showed a prominent signal at δ 22.7 ppm (Fig. 1D), in agreement with the observations of *O. sativa*. However, feeding of [26-^{13}C]desmosterol (**2**) to *Ajuga* produced cholesterol of which the ^{13}C-NMR spectra (Fig. 1C) showed ^{13}C-enriched signals at both δ 22.5 and 22.7 ppm, with the former being predominant. This partial scrambling of ^{13}C between C-26 and C-27 was further supported by a complementary experiment using [27-^{13}C]desmosterol[3] as the substrate, wherein the signal at δ 22.7 ppm was more intense than that of δ 22.5 ppm (data not shown). One possible interpretation of this scrambling is partial isomerization of **2** into **3**, followed by stereospecific reduction of the resulting mixture of **2** and **3** to give **1a** and **1b**, respectively. More definite evidence for the stereoselective *Si*-face addition at C-25 of Δ^{24}-sterol in *Ajuga* was recently obtained in a feeding experiment with $^{13}C_2$-acetate.[14] Thus the biosynthesized cholesterol indicated the ^{13}C signal at δ 22.5 ppm as a singlet, and that at δ 22.7 ppm as a flanking doublet (J=35.8 Hz). These data imply that C-26 was derived from C-2 of mevalonate *via* C-26 of desmosterol, while C-25 and -27 were derived from the intact acetate unit, and are therefore consistent with the expected hydrogen addition on the 25-*Si*-face of desmosterol.

In conclusion, it has been shown for the first time in higher plants that hydrogen introduction at C-25 during cholesterol synthesis occurs from the *Si*-face of desmosterol and from the *Re*-face of $\Delta^{25(26)}$-cholesterol.

ACKNOWLEDGMENTS We thank Drs. T. Matsumoto and N. Tanaka (Bioassay Laboratory, Research Center, Daicel Chemical Industries Ltd.) and Dr. S. Toki (National Institute of Agrobiological Resources) for providing *Ajuga* hairy root and *O. sativa* cell cultures, respectively.

REFERENCES AND NOTES

1) Voet D., Voet J. G., *Biochemistry, 2nd. ed.,* John Wiley & Sons Inc., New York, 1995, p. 699.
2) Kienle M. G., Varma R. K., Mulheirn L. J., Yagen B., *J. Am. Chem. Soc.,* **95**, 1996-2001 (1973).
3) Yagi T., Kobayashi N., Morisaki M., Hara N., Fujimoto Y., *Chem. Pharm. Bull.,* **42**, 680-681 (1994).
4) Fujimoto Y., Nagakari M., Ikuina Y., Ikekawa N., Kakinuma K., *J. Chem. Soc., Chem. Commun.,* **1991**, 688-689.
5) Seo S., Uomori A., Yoshimura Y., Tori K., *J. Chem. Soc., Perkin Trans. I,* **1984**, 869-874, and the references cited therein.
6) Ikuina Y., Kanzawa Y., Fujimoto Y., Kakinuma K., *Chem. Pharm. Bull.,* **37**, 1755-1757 (1989). The synthetic [26-^{13}C]desmosterol was enriched to more than 98% ^{13}C; 89% of the label resided at C-26 and 11% at C-27.
7) This compound (**3**) enriched to 97% ^{13}C was prepared via a Wittig reaction of a 26-nor-25-oxocholesterol derivative with $^{13}CH_2=PPh_3$. **3**: ^{1}H-NMR δ, 4.66 and 4.68 ppm(2H, a pair of d, J_{C-H}=155Hz); ^{13}C-NMR δ, 109.47 ppm.
8) Yamada J., Morisaki M., Iwai K., Hamada H., Sato N., Fujimoto Y., *Tetrahedron,* **53**, 877-884 (1997).
9) Seo S., Yoshimura Y., Satoh T., Uomori A., Takeda K., *J. Chem. Soc. Perkin Trans, I,* **1986**, 411-414.
10) Fujimoto Y., Ohoyama K., Sato N., Yamada J., Morisaki M., *Chem. Pharm. Bull.,* **45**, 224-226 (1997).
11) This result confirmed our recent conclusion (ref. 10) on the steric course of hydrogen migration from C-24 to C-25 during biosynthesis of 24-methylenecholesterol in higher plants.
12) Seo S., Uomor A., Yoshimura Y., Takeda K., Seto H., Ebizuka Y., Noguchi H., Sankawa U., *J. Chem. Soc, Perkin Trans. I,* **1989**, 1969-1974.
13) Yagi T., Morisaki M., Kushiro T., Yoshida H., Fujimoto Y., *Phytochemistry,* **41**, 1057-1064 (1996).
14) Nakagawa T., Hara N., Fujimoto Y., *Tetrahedron Lett.,* in press.

(Received February 12 , 1997; accepted March 25, 1997)