^{13}C ASSIGNMENT OF DIASTEREOTOPIC C-26 AND -27 METHYL GROUPS OF 24-METHYLENECHOLESTEROL: STERIC COURSE OF HYDROGEN MIGRATION FROM C-24 TO C-25 DURING ITS BIOSYNTHESIS IN HIGHER PLANTS

Yoshinori FUJIMOTO,*,[a] Kiyoshi OHYAMA,[a] Naoko SATO,[a] Junko YAMADA,[b] and Masuo MORISAKI[b]

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

Chemical shifts of the diastereotopic methyl groups (C-26 and C-27) of 24-methylenecholesterol have been unambiguously assigned by synthesizing stereochemically defined ^{13}C-labeled compounds. This allowed us to establish the steric course of hydrogen migration from C-24 to C-25 during the formation of 24-methylenecholesterol from a Δ^{24}-precursor in a tissue culture of *Catharanthus roseus*.

KEY WORDS plant sterol; stereochemistry; sterol biosynthesis; prochirality; Δ^{24}-sterol methyl transferase; *Catharanthus roseus*

Plant sterols are characterized by a C-24 alkyl substituent (methyl, ethyl, methylene, or ethylidene) which arises from *S*-adenosylmethionine by a simple or double trans-methylation reaction on an olefinic precursor. 24-Methylenesterols, such as 24-methylenecholesterol (**1**) and 24-methylenecycloartanol (**2**), are the first intermediates of plant sterol biosynthesis produced by the transfer of a methyl group on a $\Delta^{24(25)}$-precursor in higher plants. The formation of 24-methylene-sterol involves hydrogen migration from the C-24 to C-25 position and loss of one hydrogen atom from the methyl group originating from *S*-adenosylmethionine (Chart 1).[1] From a stereochemical point of view, two stereogenic centers are generated at the C-24 and C-25 positions during this methylation step and they are controlled by Δ^{24}-sterol methyl transferase.[2] The direction of the transfer of the methyl group, either *Re*-face or *Si*-face attack on C-24 of the $\Delta^{24(25)}$-precursor, determines the stereochemistry of the C-24 position of the C-25 cationic intermediate, although it disappears during the formation of 24-methylenesterol. *Si*-face attack at C-24 (24β-orientation of the methyl group) has been proposed in higher plants[3] and yeast.[4]

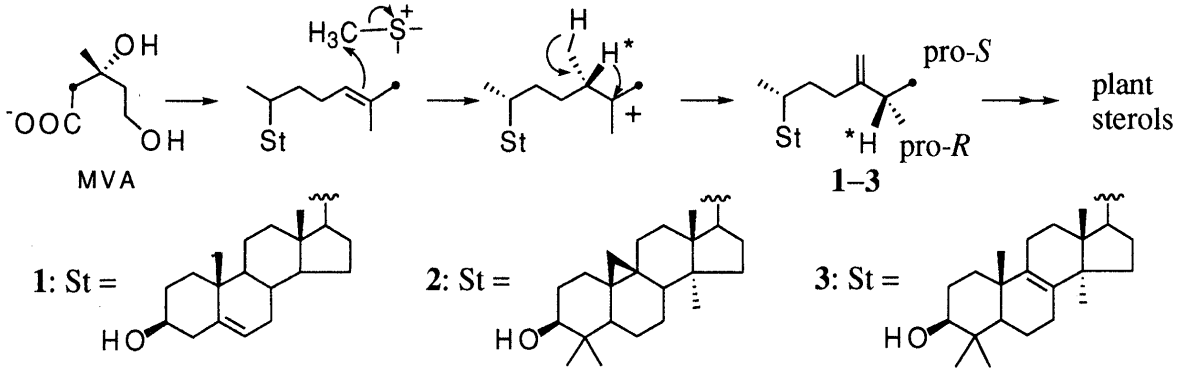

Chart 1. Mechanism of 24-Methylenecholesterol Biosynthesis in Higher Plants
The dots refer to the carbon correlated to C-2 of mevalonate

* To whom correspondence should be addressed.

© 1997 Pharmaceutical Society of Japan

NII-Electronic Library Service

The steric course of the hydrogen migration from the C-24 to C-25 position determines the metabolic fate of the C-26 and -27 methyl groups of $\Delta^{24(25)}$-sterol and the C-25 prochilarity of the resulting 24-methylenesterol. In higher plants, *Re*-face hydrogen migration was tentatively proposed in the formation of 24-methylenecholesterol (**1**) in *Physalis perviana*[5] and 24-methylencycloartanol (**2**) in *Trichosanthes kirilowii*.[6] The same steric course was suggested for the biosynthesis of poriferasterol in *Ochromonas malhamensis*,[7] while *Si*-face hydrogen migration to C-25 was reported in the formation of isofucosterol in *Pinus pinea*.[8] In ergosterol biosynthesis in yeast, *Re*-face hydrogen migration has been established.[9] However, the ^{13}C chemical shifts of the diastereotopic methyl groups (C-26 and C-27) on C-25 of 24-methylenesterol have not been rigorously assigned in any of these previous works. Quite recently, Nes and his coworkers reported the operation of *Re*-face hydrogen migration in corn, *Zea mays*, by isolating 24(28)-methylene-24,25-dihydrolanosterol (**3**), whose C-25 prochirality was indirectly deduced from its biological conversion into ergosterol.[10] Their paper prompted us to report our independent work on the stereochemistry of hydrogen migration in cultured cells of *Catharanthus roseus*, which features unambiguous stereochemical assignments at C-25 of 24-methylenecholesterol itself, based on the synthesis of the stereochemically defined pro-*R*- and pro-*S*-methyl-^{13}C-labeled 24-methylenecholesterols (**1a** and **1b**) and their ^{13}C-NMR analysis.

We previously assigned the ^{13}C chemical shifts of the pro-*R*- and pro-*S*-methyl groups on C-25 of fucosterol and isofucosterol.[11] The same method (Chart 2) was used in the present study. Thus, the previously prepared[11] ^{13}C-labeled sterols with the known C-25 configuration, (pro-*R*-methyl)-^{13}C-labeled ketone (**4a**) and the (pro-*S*-methyl)-^{13}C-labeled ketone (**4b**), were submitted to a Wittig reaction to give the 24(28)-olefin TBS ethers, which on deprotection of the TBS group, furnished the (pro-*R*-methyl)-^{13}C-labeled (**1a**) and (pro-*S*-methyl)-^{13}C-labeled (**1b**) 24-methylenecholesterols, respectively, in 75% yield for the two steps. The partial ^{13}C-NMR spectra of **1a** and **1b** are shown in Fig. 1, which clearly indicate that the signal resonating at the higher field (δ 21.85) is assigned to the pro-*S*-methyl group and the signal at the lower field (δ 21.98) to the pro-*R*-methyl group.[12]

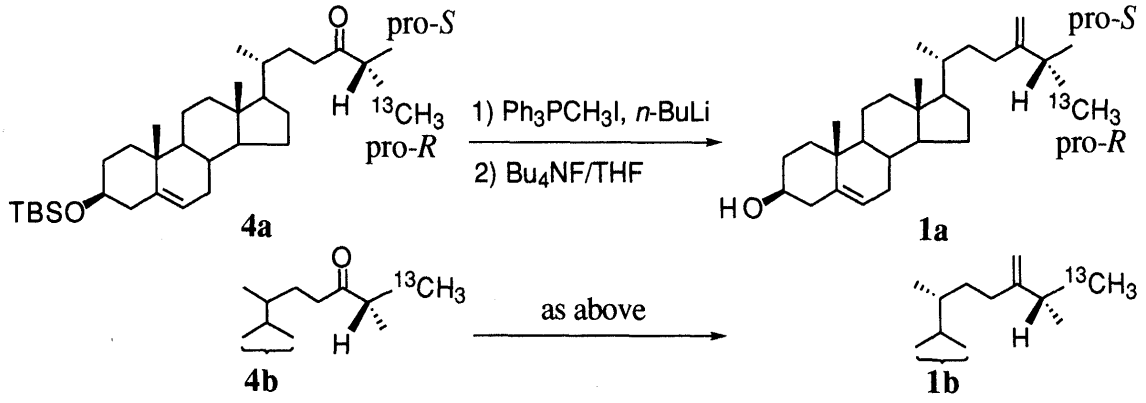

Chart 2. Synthesis of Stereospecifically ^{13}C-Labeled 24-Methylenecholesterols (**1a** and **1b**)

With these unambiguous assignments in hand, we then carried out a feeding experiment of ^{13}C-desmosterol labeled at the (*E*)-methyl group at C-25[13] with the cultured cells of *C. roseus*, which is known to produce 24-methylenecholesterol under feeding conditions.[14] The sterol fraction was separated and the 24-methylenecholesterol fraction was finally purified by HPLC as described previously.[14] The partial ^{13}C-NMR spectrum of the 24-methylenecholesterol fraction is also included in Fig. 1. The higher-field carbon (pro-*S* methyl at C-25) of 24-methylenecholesterol was found to be enriched. The results indicate that *Re*-face hydrogen migration takes place in cultured *Catharanthus* cells.

NII-Electronic Library Service

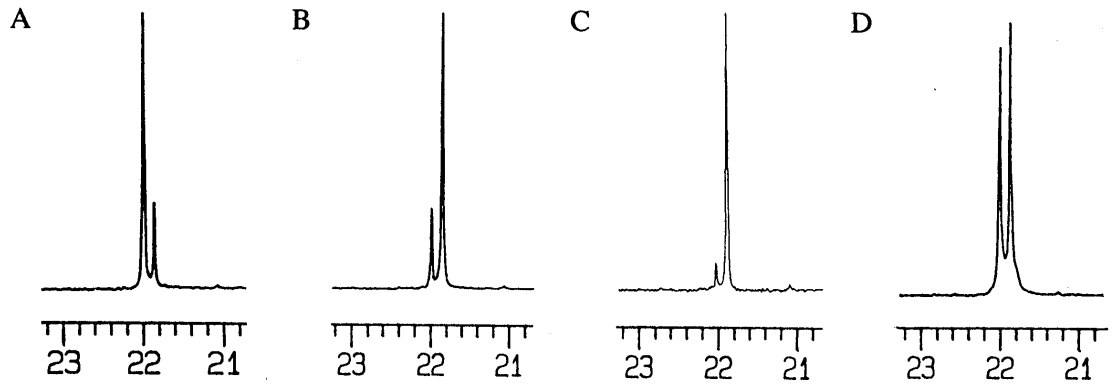

Fig. 1. Partial ^{13}C-NMR ($CDCl_3$) Spectra of 24-Methylenecholesterol
A, chemically prepared Pro-*R*-Me-^{13}C-labeled **1a**; B, Pro-*S*-Me-^{13}C-labeled **1b**; C, metabolically produced **1b** from (*E*)-Me-^{13}C-labeled desmosterol; D, metabolically produced **1a**+**1b** from [26,27-$^{13}C_2$]desmosterol.[14]

The steric course established in the present studies agrees with those proposed to occur in other higher plants.[5,6,10] It can be now generalized that the formation of 24-methylenesterols in higher plants proceeds *via* the *Re*-face hydrogen migration mechanism. Thus, the fate of the diastereotopic methyl groups on C-25 can be depicted as shown in Chart 1, wherein the (*E*)-methyl group of $\Delta^{24(25)}$-precursor originating from C-2 of mevalonate becomes the pro-*S*-methyl group of 24-methylenesterol.

ACKNOWLEDGMENT We thank Prof. Hiroki Hamada, Okayama Science University, for providing tissue cultures of *C. roseus*.

REFERENCES AND NOTES

1) Nes W. R., McKean M. L., "*Biochemistry of Steroids and Other Isoprenoids*;" University Park Press, Baltimore, 1977, pp. 325-410.
2) Nes W. D., Janssen G. G., Bergenstrahle A., *J. Biol. Chem.*, **266**, 15202-15212 (1991).
3) Yagi T., Morisaki M., Kushiro T., Yoshida H., Fujimoto Y., *Phytochemistry*, **41**, 1057- 1064 (1996).
4) Arigoni D., *Ciba Found. Symp.*, **60**, 243-261 (1978).
5) Seo S., Uomori A., Yoshimura Y., Takeda K., *J. Chem. Soc., Chem. Commun.*, **1984**, 1174-1176.
6) Seo S., Uomori A., Yoshimura Y., Sankawa U., Ebizuka Y., Seto H., Takeda K., *J. Chem. Soc., Perkin Trans. 1*, **1987**, 1876-1878.
7) Nicotra F., Ronchetti F., Russo, G., Toma L., *Magnetic Resonance Chem.*, **23**, 134-136 (1985); the steric course in this paper was erroneously reported as *Si*-face migration based on the incorrect assignment of the diastereotopic methyl groups.
8) Nicotra F., Ronchetti F., Russo, G., Lugaro G., Casellato M., *J. Chem. Soc., Perkin Trans. 1*, **1981**, 498-502.
9) Zhou W., Guo D., Nes W. D., *Tetrahedron Lett.*, **1996**, 1339-1342; references cited therein.
10) Guo D., Jia Z., Nes W. D., *J. Am. Chem. Soc.*, **118**, 8507-8508 (1996).
11) Fujimoto Y., Ikuina Y., Nagakari M., Kakinuma K., Ikekawa N., *J. Chem. Soc., Perkin Trans. 1*, **1991**, 2041-2046.
12) The assignments eventually coincide with those reported in references 5, 6, and 10.
13) Ikuina Y., Kanzawa Y., Fujimoto Y., Kakinuma K., *Chem. Pharm. Bull.*, **37**, 1755-1758 (1989).
14) Yamada J., Morisaki M., Iwai K., Hamada H., Sato N., Fujimoto Y., *Tetrahedron*, in press.

(Received October 30, 1996; accepted November 21, 1996)

NII-Electronic Library Service