Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 205–208

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

STEREOCHEMISTRY OF THE REDUCTION OF 24-ETHYLDESMOSTEROL TO SITOSTEROL IN TISSUE CULTURES OF *ORYZA SATIVA*

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Received 1 October 1997; accepted 1 December 1997

Abstract: Feeding of [26-^{13}C]- and [27-^{13}C]-24-ethyldesmosterols to cultured cells of *Oryza sativa* followed by ^{13}C-NMR analysis of the biosynthesized sitosterol revealed that the reduction of 24(25)-double bond proceeds with an *anti*-addition of hydrogen atoms, thus the *E*-methyl group of the olefinic precursor becomes the pro-*S*-methyl on C-25 of sitosterol.

In the biosynthesis of side-chain of sitosterol (**1**), one of typical higher plant sterols, its 24*R* stereochemistry is determined by the final reduction step of a 24(25)-olefinic sterol such as 24-ethyldesmosterol (**2**), which is produced by the double-bond isomerization of a 24(28)-olefinic sterol such as isofucosterol (**3**). Isofucosterol is formed from a 24(28)-olefinic sterol such as 24-methylenecholesterol (**4**) by the transfer of a methyl group from *S*-adenosylmethionine (Fig. 1).[1] We have recently demonstrated that 24-ethyldesmosterol is converted into sitosterol with cultured cells of *Oryza sativa*.[2] Further, we have reported on the stereochemistry of the reduction of 24-methyldesmosterol affording campesterol and dihydrobrassicasterol in the same cell cultures.[3] The stereochemistry of the reduction of 24-ethyldesmosterol to sitosterol is described herein.

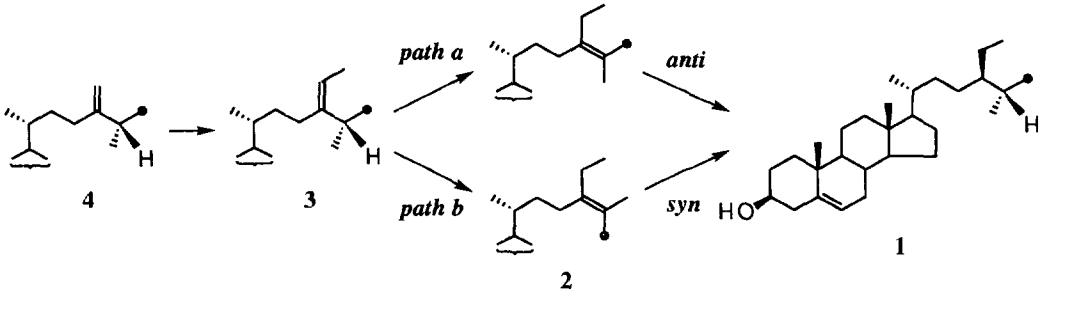
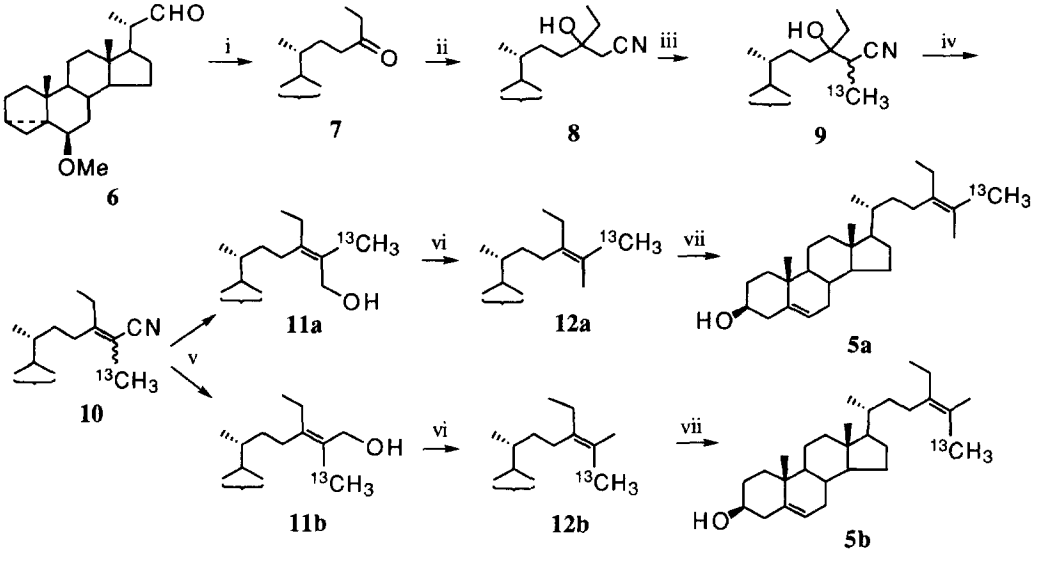


Fig. 1 Two possible pathways in the formation of sitosterol side chain.
Dots designate the carbons derived from C-2 of mevalonate.

Concerning the metabolic origins of C-26 and C-27 of **1**, **2**, **3** and **4**, it is well established that the pro-*S* pro-*R* methyl groups on C-25 of 24-methylenecholesterol (**4**)[4] and sitosterol (**1**)[5] are derived from C-2 and C-6 of mevalonate, respectively. The same seems to be the case for isofucosterol (**3**) in *Physalis peruviana*[6] and *Catharanthus roseus*,[7] while in *Pinus pinea* the reversed origin was reported.[8] However, little has been known

0960-894X/98/$19.00
PII: S0960-894X(97)10220-7

about the origin of the isopropyliden (*E*)- and (*Z*)-methyl groups of 24-ethyldesmosterol (**2**), although this sterol has been characterized as a minor sterol constituent from several higher plants.[9] Thus, two metabolic pathways remain to be clarified in the conversion of isofucosterol to sitosterol; *path a* wherein the pro-*S* methyl of **3** becomes (pro-*S*)-methyl of **1** *via* the (*E*)-methyl of **2**, and *path b* wherein the (*Z*)-methyl of **2** corresponds to C-2 of mevalonate. To differentiate the two pathways, the fate of (*E*)- and (*Z*)-methyl groups of **2** was chased by feeding regiospecifically ^{13}C-labeled compounds to cultured cells of *Oryza sativa*.



Reagent and conditions: i) LDA, acetone; MsCl, Et_3N; DBU; H_2, Pd-C (50%); ii) LDA, CH_3CN (98%); iii) LDA, $^{13}CH_3I$ (61%); iv) $SOCl_2$ (100%); v) DIBAL leading to alhehyde; DIBAL leading to **11** (31% for **11a** and 31% for **11b**); vi) MsCl, LiCl, lutidine; $LiAlH_4$ (72%); vii) TsOH/H_2O (96%).

Scheme 1 Synthesis of regiospecifically ^{13}C-labeled 24-ethyldesmosterols (**5a**) and (**5b**)

The requisite labeled sterols, [26-^{13}C]-24-ethyldesmosterol (**5a**) (C-26 refers to (*E*)-methyl group) and [27-^{13}C]-24-ethyldesmosterol (**5b**), were synthesized essentially in the same manner as described previously (Scheme 1).[3] Ethyl ketone **7**, obtained from well known steroidal C-22 aldehyde **6**,[10] was reacted with acetonitrile anion to give adduct **8**. Metylation of the adduct using $^{13}CH_3I$ (99% ^{13}C) afforded ^{13}C-labeled24-ol **9** which was dehydrated to give a mixture of (*E*)- and (*Z*)-tetrasubstituted olefin **10**. Stepwise reduction of **10** with DIBAL *via* the corresponding alhehyde gave a mixture of allylic alcohols **11a,b**. The geometric isomers were separated by silica gel Lobar column, affording the less polar (*E*)-alcohol **11a** (δ_C 15.84) and the more polar (*Z*)-isomer **11b** (δ_C 16.00). The (*Z*)-geometry of **11b** was determined by NOE studies in which irradiation of oxymethylene signal (δ 4.09, d, *J*=4.6 Hz) caused the enhancement of the signal intensity of 28-H_2 resonance (δ 2.08, q, *J*=7.4 Hz). The alcohols **11a** and **11b** were converted into the (*E*)-Me- and (*Z*)-Me-^{13}C-labeled sterols (**5a**, δ_C 19.97) and (**5b**, δ_C 20.11), *via* **12a** and **12b**, respectively.

Feeding experiments of **5a** (50 mg) to cultured cells of *O. sativa* was carried out as described previously.[2] The cultures were incubation for 2 weeks under dark when the cells were collected from which a sterol fraction

(59 mg) was obtained. GLC analysis indicated that the fraction is a mixture of campesterol, stigmasterol, sitosterol and the substrate. The ^{13}C-NMR spectrum of the fraction showed an enriched signal at δ 19.82,[11] assignable to the pro-*S*-Me of **1**, together with an intense signal due to the recovered **5a**. Sitosterol was separated by reversed-phase HPLC and ^{13}C-MNR was recorded. The spectrum (Fig. 2) clearly showed that the signal of pro-*S* of **1** was enriched. Feeding of **5b** (50 mg) gave the complementary result of the pro-*R* methyl group (δ 19.02) of the biosynthesized sitosterol being enriched with ^{13}C, as indicated in Fig. 2.

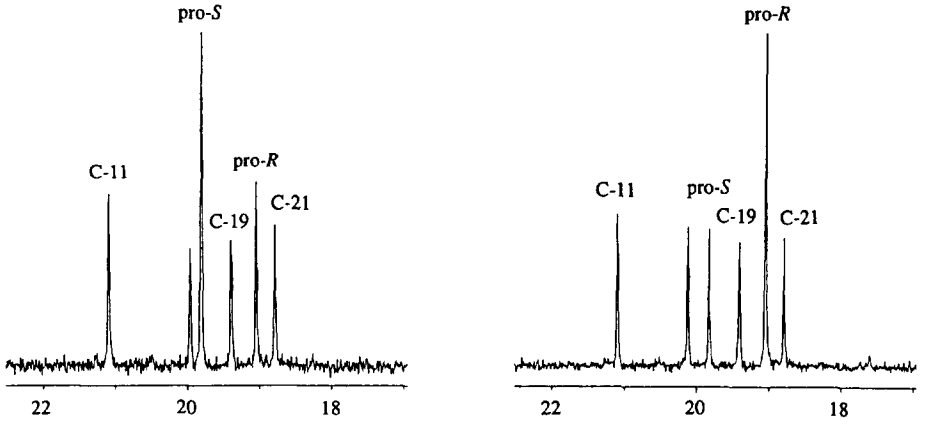


Fig. 2 Partial ^{13}C-NMR spectra (75 MHz, $CDCl_3$) of the purified sitosterol derived from **5a** (left) and **5b** (right). The peaks at δ 19.97 and 20.11 are due to the (*E*) and (*Z*)-Me groups of unremoved substrate, respectively.

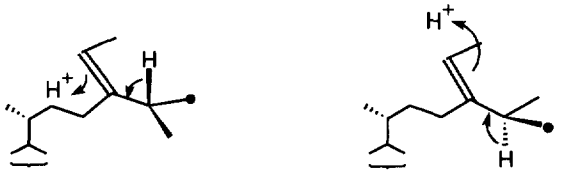


Fig. 3 Two possible conformations in the step of the double bond isomerization from 24(28) to 24(25). Orientation of the protonation at C-28 is tentative.

These data clearly indicate that the (*E*)-methyl group of 24-ethyldesmosterol becomes the pro-*S*-methyl of sitosterol, whereas the (*Z*)-methyl group turns to the pro-*R*-methyl. It implies that the reduction takes place with an anti-addition of hydrogen atoms from 24-*Si* and 25-*Re* face. Thus, it is concluded that path *a* (Fig. 1) is operating in tissue cultures of *O. sativa*. The step of double-bond isomerization (**3** → **2**) would take conformation *A*, rather than conformation B (Fig. 3) to satisfy the fate of (*E*)- and (*Z*)-methyl groups of **3**. These steric courses are consistent with that recently established for the formation of campesterol in the same cultures.[3] We are inclined to think that the metabolic relationships of C-26 and -27 as illustrated in Fig. 1 (path *a*) is general for sistosterol-producing higher plants, and the reported observation with *P. pinea*[8] seems to be exceptional.

ACKNOWLEDGMENT We thank Dr. Seiichi Toki, National Institute of Agrobiological Resources, for providing tissue cultures of *O. sativa*.

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