Stereochemistry of the reduction of 24-methyldesmosterol to campesterol and dihydrobrassicasterol in higher plants

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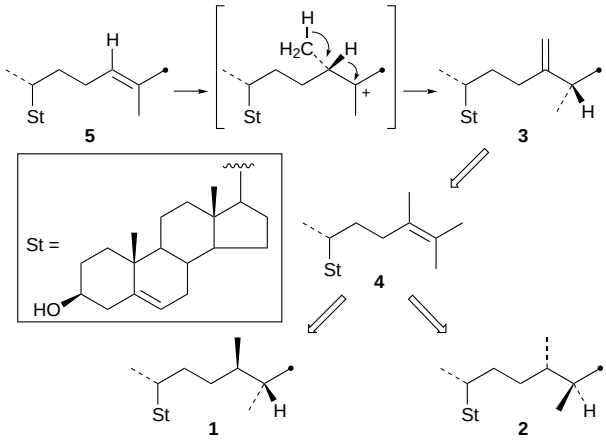
Feeding of chemically synthesized [26-^{13}C]- and [27-^{13}C]-labelled 24-methyldesmosterols to tissue cultures of *Oryza satiba* and *Catharanthus roseus* followed by ^{13}C NMR analysis of the biosynthesized sterols reveals that reduction of the 24(25)-double bond giving either campesterol or dihydrobrassicasterol takes place in an *anti*-manner.

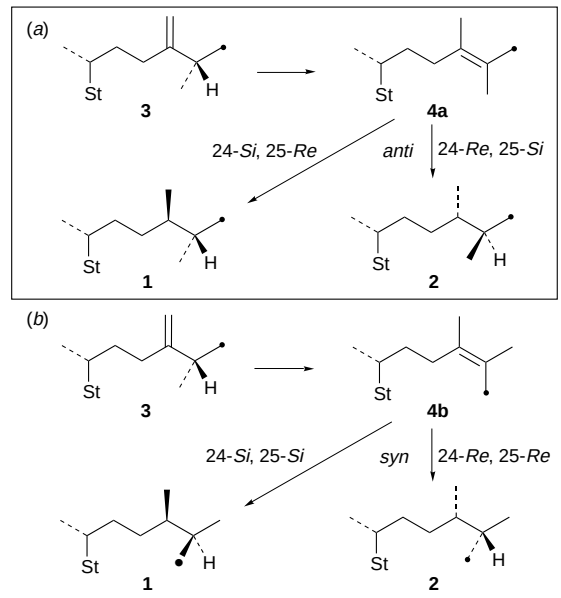
Plant sterols are characterized by a C-24 alkyl substituent (methyl, ethyl, methylene or ethylidene) which originates from *S*-adenosylmethionine by a single or double *trans*-methylation reaction with an olefinic precursor.[1] Accumulated evidence suggests that the final step of the side chain biosynthesis of 24-methylsterols, campesterol **1** and dihydrobrassicasterol **2**, in higher plants is the reduction of 24-methyl-$\Delta^{24(25)}$-sterol (*e.g.* 24-methyldesmosterol **4**).[1,2] The $\Delta^{24(25)}$-sterol is proposed to be formed by the isomerization of 24-methyl-$\Delta^{24(28)}$-sterol (*e.g.* 24-methylenecholesterol **3**) which in turn is produced by the transfer of the methyl group of *S*-adenosylmethionine to a $\Delta^{24(25)}$-sterol such as desmosterol **5** (Scheme 1).[1] Similar reduction of 24-ethyl-$\Delta^{24(25)}$-sterol is proposed for sitosterol biosynthesis. Recently, we offered evidence supporting an intermediary role of 24-methyl-$\Delta^{24(25)}$-sterol in the biosynthesis of campesterol as well as dihydrobrassicasterol in tissue cultures of *Catharanthus roseus* and *Oryza sativa*.[3] Furthermore, we assigned the ^{13}C NMR chemical shifts of the C-26 and -27 diastereotopic methyl groups of **3** and showed that the isopropylidene (*E*)- and (*Z*)-methyl groups of **5** *pro-S* and *pro-R* methyl groups of **3**, respectively, in the above two tissue cultures.[4] The same steric course in the conversion of lanosterol into 24-methylenelanosterol was reported with *Zea mays*.[5] On the other hand, the C-2 of mevalonate [*via* (*E*)-methyl group of **5**] turns the *pro-S* methyl group of **1** and *pro-R* methyl groups of **2**, whereas C-6 of mevalonate [*via* the (*Z*)-methyl group of **5**] becomes the *pro-R* methyl group of **1** and *pro-S* methyl groups of **2** with cultured cells of *Physalis perviana*[6] and *Amsonia elliptica*.[2] We also obtained the same results on the origin of the diastereotopic methyl groups of **1** and **2** by feeding (*E*)- and (*Z*)-Me ^{13}C-labelled desmosterols to cultured cells of *C. roseus* and *O. sativa*. Thus, the biosynthetic pathway of 24-methylcholesterols in higher plants can be summarized as depicted in Scheme 1.



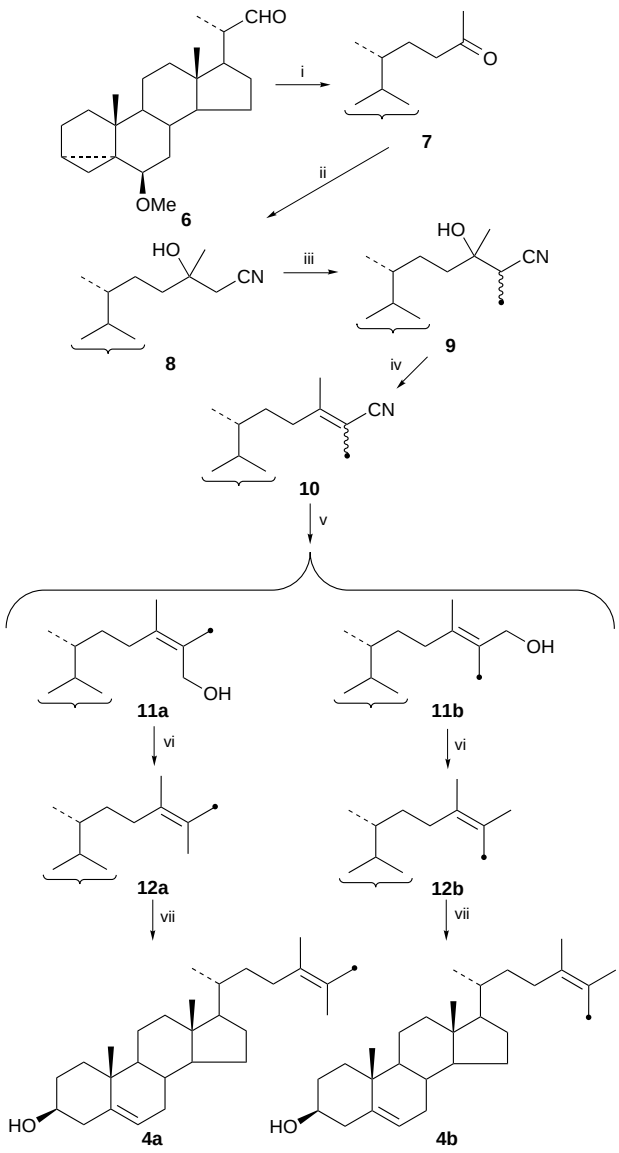
Scheme 1 Proposed biosynthetic pathway of 24-methylcholesterols. The dots indicate the carbon derived from C-2 of mevalonate.

The steric course of the isomerization from $\Delta^{24(28)}$ to $\Delta^{24(25)}$ (**3** → **4**) and the stereochemistry of the subsequent reduction of $\Delta^{24(25)}$ (**4** → **1** and **2**) remain unclear. Two possible mechanisms can be drawn for the conversion of **3** to **1** and **2**: (*a*) *pro-S*-Me at C-25 of **3** becomes (*E*)-Me of **4** in the double bond isomerization reaction and the reduction proceeds in an *anti*-mode; (*b*) *pro-S*-Me at C-25 of **3** becomes (*Z*)-Me of **4** and the reduction occurs in a *syn*-mode (Scheme 2). We now report that mechanism (*a*) operates in tissue cultures of *O. sativa* and *C. roseus*.

(*E*)-Me ^{13}C-labelled **4a** and (*Z*)-Me ^{13}C-labelled **4b** 24-methyldesmosterols were prepared (Scheme 3). Methyl ketone **7** was obtained from well known C-22-aldehyde **6**[7] in four steps. Reactions of **7** with the MeCN anion gave adduct **8**. Methylation of the adduct using $^{13}CH_3I$ (99% ^{13}C) afforded ^{13}C-labelled **9** which was dehydrated to give a mixture of (*E*)- and (*Z*)-tetrasubstituted olefin **10**. Stepwise reduction of the olefin mixture with DIBAL-H *via* the corresponding aldehyde gave a mixture of allyic alcohols **11a**,**b**. The geometric isomers were separated by a silica gel Lobar column, affording the less polar (*E*)-alcohol **11a** and the more polar (*Z*)-isomer **11b**. The (*E*)-geometry of **11a** was determined by NOE studies in which irradiation of 28-H_3 (δ 1.67) caused the enhancement of the signal intensity of the CH_3 resonance at δ 1.73 (d, J = 126 Hz). Similarly, irradiation of 28-H_3 (δ 1.71) of **11b** resulted in the enhancement of the intensity of oxymethylene proton signal at



Scheme 2 Two possible routes for the formation of 24-methylcholesterol



Scheme 3 Synthesis of regiospecifically labelled 24-methyl-$\Delta^{24(25)}$-cholesterols **4a** and **b**. *Reagent and conditions*: i, acetone, LDA, then MsCl, Et_3N, then DBU, then H_2, Pd–C (50%); ii, LDA, MeCN (91%); iii, LDA, $^{13}CH_3I$ (41%); iv, $SOCl_2$ (100%); v, DIBAL-H (19% **11a**, 25% **11b**); vi, MsCl, LiCl, lutidine; LAH (70%); vii, TsOH, H_2O (80%).

δ 4.11 (d, J = 4.6 Hz), thus establishing the (*Z*)-geometry for **11b**. The alcohols **11a**,**b** were converted into the (*E*)-Me and (*Z*)-Me ^{13}C-labeled sterols **4a** (δ_C 20.51) and **4b** (δ_C 19.98), respectively, *via* **12a** and **12b**.

Feeding of **4a** to cultured cells of *O. sativa* was carried out as described previously.[3] HPLC separation of the resulting sterol fraction gave a mixture of campesterol and dihydrobrassicasterol. The partial ^{13}C NMR spectrum of the mixture is shown in Fig. 1. Compound **4b** was similarly incubated to give the same 24-methylcholesterol mixture (Fig. 1).

^{13}C Assignments of the diastereotopic methyl groups of **1** and **2** were established previously.[8] Thus, it is evident from Fig. 1 that the (*E*)-methyl of **4** becomes the *pro-S*-methyl of **1** and the *pro-R*-methyl of **2**, whereas the (*Z*)-methyl of **4** becomes the *pro-R*-methyl group of **1** and the *pro-S*-methyl group of **2**. Similar feeding experiments of **4a**,**b** using cultured cells of *C. roseus* led to the same results for the metabolic fates of the isopropylidene methyl groups (data not shown).

These findings demonstrate for the first time that stereospecific hydrogen attack on 24-*Si*, 25-*Re* face of 24-methyldesmosterol **4** affords campesterol **1**, whereas dihydrobrassicasterol **2** is produced by 24-*Re*, 25-*Si* attack of hydrogen on the same 24(25)-olefin **4**. It should be noted that an *anti*-mode of hydrogen addition occurs in both cases.

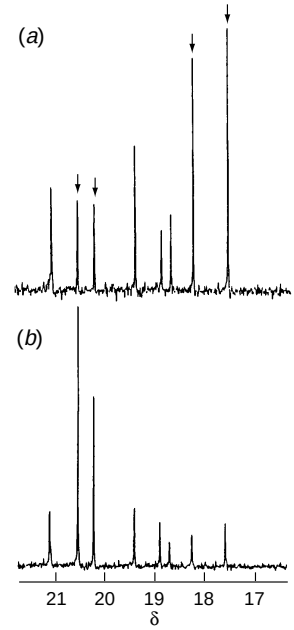


Fig. 1 ^{13}C NMR spectra (part) of metabolically formed 24-methylcholesterol fractions. (*a*) [(*E*)-Me-^{13}C]-**4a**, (*b*) [(*Z*)-Me-^{13}C]-**4b**. Signals indicated by arrows: *pro-S*-Me (δ 18.26) and *pro-R*-Me (δ 20.19) of **1**, *pro-S*-Me (δ 20.50) and *pro-R*-Me (δ 17.60) of **2**.

These results imply that the double bond migration from **3** to **4** should proceed in such a manner that the *pro-R* methyl of **3** becomes (*Z*)-methyl group of **4** while the *pro-S* methyl of **3** becomes (*E*)-methyl group of **4** [Scheme 2(*a*)]. The metabolic correlation of C-26 and C-27 in 24-methylcholesterol biosynthesis in *O. sativa* and *C. roseus* can be summarized as: C-2 of mevalonate → (*E*)-Me of **5** → *pro-S*-Me of **3** → (*E*)-Me of **4** → *pro-S*-Me of **1** and *pro-R*-Me of **2**; C-6 of mevalonate → (*Z*)-Me of **5** → *pro-R*-Me of **3** → (*Z*)-Me of **4** → *pro-R*-Me of **1** and *pro-S*-Me of **2** (Scheme 1).

It has been reported that one of the olefinic methyl groups of **4**, which appeared at higher field [δ 19.93, now assigned to (*Z*)-methyl group] in the ^{13}C NMR spectrum, was derived from an intact [$^{13}C_2$]acetate molecule with tissue cultures of *P. periviana*,[6] which coincides with our present results.

Footnote

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