Stereospecificity in the Side-chain Formation of 24-Methyldesmosterol in Cell Cultures of *Physalis peruviana*

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24-Methyldesmosterol isolated from cell cultures of *Physalis peruviana* fed with [1,2-$^{13}C_2$]acetate is shown to be stereospecifically labelled at C-26 and C-27.

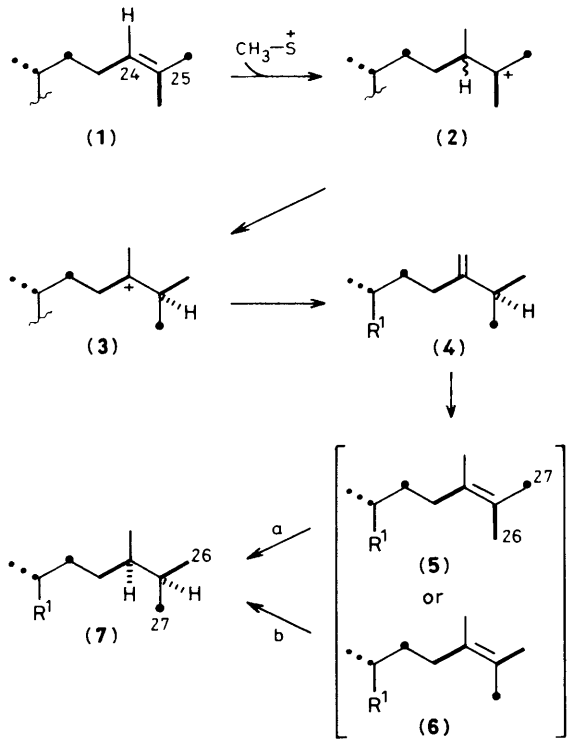
The participation of a $\Delta^{24(25)}$ sterol in the biosynthesis of 24α-alkylsterols has been suggested.[1] We observed previously that the hydrogen atom at C-24 of the Δ^{24}-precursor (**1**), arising from [2-^{14}C, (4*R*)-4-$^{3}H_1$]mevalonic acid (MVA), is lost during the side-chain formation of a 24α-ethylsterol, stigmasterol, in cultured cells of *Nicotiana tabacum* and *Dioscorea tokoro*,[2] assuming the intermediacy of a $\Delta^{24(25)}$-sterol. Sitosterol, one of the most common 24α-ethylsterols, was also suggested to be biosynthesized *via* a process involving isomerization of the 24(28) double bond of isofucosterol to the 24(25) double bond prior to the reduction yielding the saturated side chain in *Holdeum vurgare*.[3] We recently demonstrated that the isomerization and reduction of the double bond take place stereospecifically leading to sitosterol which has C-26 (*pro-R* methyl group at C-25) originating from C-2 of MVA in cultured higher plant cells.[4]

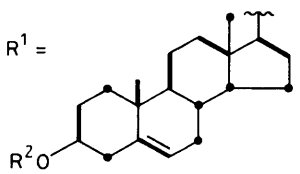
In the biosynthesis of the 24α-methylsterol, campesterol (**7**), a $\Delta^{24(25)}$-intermediate, (**5a**) or (**6a**), has also been proposed to be formed by similar enzymatic isomerization of the 24(28) double bond in *Zea mays*.[5] We report here that the $\Delta^{24(25)}$-sterol, 24-methyldesmosterol, (**5a**) or (**6a**), is formed enzymatically in cultured cells of *Physalis peruviana* based on

Table 1. ^{13}C N.m.r. data of 24-methyldesmosteryl acetate (**5b**) or (**6b**) from [1,2-$^{13}C_2$]acetate in cultured *P. peruviana* cells (δ_C/p.p.m. and J_{CC}/Hz).[a]

	δ_C	*J*		δ_C	*J*		δ_C	*J*		δ_C	*J*
C-1	36.87	s	C-8	31.75	s	C-15	24.23	s	C-22	34.19	s
C-2	27.68	36	C-9	49.85	34	C-16	28.08	34	C-23	30.98	44
C-3	73.71	36	C-10	36.46	35	C-17	55.62	34	C-24	127.91	44
C-4	37.99	s	C-11	20.96	34	C-18	11.81	s	C-25	122.86	44
C-5	139.14	72	C-12	39.57	36	C-19	19.25	35	C-26	20.48[b]	s
C-6	122.19	72	C-13	42.18	36	C-20	35.84	35	C-27	19.93[b]	44
C-7	31.75	s	C-14	56.46	s	C-21	18.74	35	C-28	18.42	—
CH_3CO	21.35 and 169.88										

[a] Data were obtained on a Varian XL-200 n.m.r. spectrometer at 50.309 MHz and *ca.* 0.1 M in [^{2}H]chloroform at 24 °C. The acquisition time was 1.766 s. Accuracies of δ_C (downfield from internal Me_4Si) and J_{CC} were within 0.02 p.p.m. and 1 Hz, respectively. Multiplicity, s: singlet. [b] Assignments may be reversed.





a; $R^2 = H$
b; $R^2 = COCH_3$

the ^{13}C-labelling patterns of C-26 and C-27 (*Z*- and *E*-methyl groups, respectively) of (**5a**) or (**6a**) biosynthesized from [1,2-$^{13}C_2$]acetate.

Cell cultures of *P. peruviana* were grown in Linsmaier–Skook medium including sodium [1,2-$^{13}C_2$]acetate (200 mg/ml of 2 : 1 mixture of unlabelled and labelled acetate) for 4 weeks. 24-Methylenecholesterol (**4**) was removed by h.p.l.c. (Develosil ODS) from the phytosterol mixture to avoid the formation of the double bond migration product during the isolation procedure. Acetyl 24-methyldesmosterol (**5b**) or (**6b**) was isolated by argentation t.l.c. after acetylation of the rest of the sterol mixture. The distribution of ^{13}C-carbons from [1,2-$^{13}C_2$]acetate was analysed using the ^{13}C 'INADEQUATE' n.m.r. method.[6] The ^{13}C signal assignments and the labelling patterns are listed in Table 1. A signal at δ_C 19.93 (C-27 or C-26) clearly appeared as a doublet (J_{CC} 44 Hz) originating from C-6 of MVA and a signal at δ_C 20.48 (C-26 or C-27) appeared as a singlet arising from C-2 of MVA. These findings show that the 24(25) double bond is formed enzymatically, probably by stereospecific double bond isomerization from 24(28) to 24(25). Reduction of the 24(25) double bond in (**5a**) or (**6a**) seems to proceed stereospecifically by (C-24*si*–C–25*re*) attack (path a) or (C-24*si*–C-25*si*) attack (path b) of hydrogen atoms to lead to campesterol (**7**), which was reported in the preceding paper to have C-26 originating from C-6 of MVA in the same cultured cells.[7]

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