

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

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24-Methylidesmosterol isolated from cell cultures of *Physalis peruviana* fed with [1,2-¹³C₂]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 24 α -alkylsterols has been suggested.¹ We observed previously that the hydrogen atom at C-24 of the Δ^{24} -precursor (**1**), arising from [2-¹⁴C, (4*R*)-4-³H₁]mevalonic acid (MVA), is lost during the side-chain formation of a 24 α -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 24 α -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

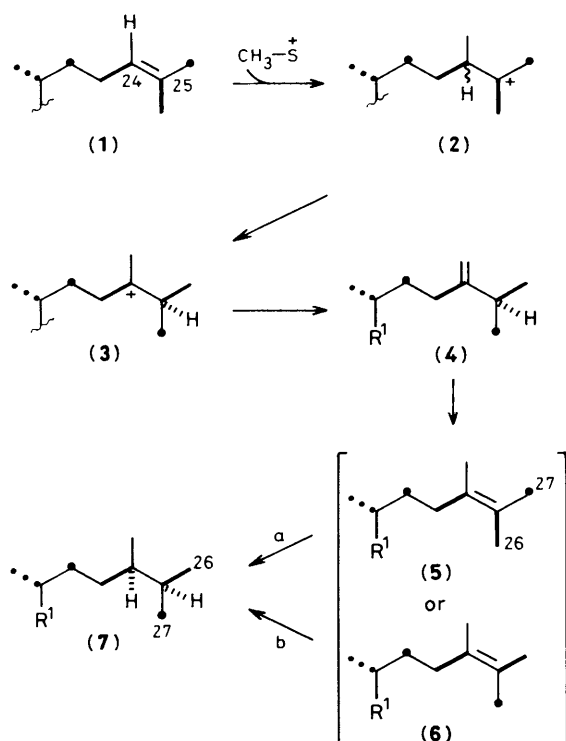
saturated side chain in *Holdeum vurgare*.³ We recently demonstrated that the isomerization and reduction of the double bond take place stereospecifically leading to sitosterol which has C-26 (*pro-R* methyl group at C-25) originating from C-2 of MVA in cultured higher plant cells.⁴

In the biosynthesis of the 24 α -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-methylidesmosterol, (**5a**) or (**6a**), is formed enzymatically in cultured cells of *Physalis peruviana* based on

Table 1. ¹³C N.m.r. data of 24-methylidesmosteryl acetate (**5b**) or (**6b**) from [1,2-¹³C₂]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 ₃ CO	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 [²H]chloroform at 24 °C. The acquisition time was 1.766 s. Accuracies of δ_C (downfield from internal Me₄Si) and J_{CC} were within 0.02 p.p.m. and 1 Hz, respectively. Multiplicity, s: singlet. ^b Assignments may be reversed.



the ^{13}C -labelling patterns of C-26 and C-27 (*Z*- and *E*-methyl groups, respectively) of (5a) or (6a) biosynthesized from $[1,2-^{13}\text{C}_2]$ acetate.

Cell cultures of *P. peruviana* were grown in Linsmaier-Skoog medium including sodium $[1,2-^{13}\text{C}_2]$ 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-methylcholesterol (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 $[1,2-^{13}\text{C}_2]$ 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 $\delta_{\text{C}} 19.93$ (C-27 or C-26) clearly appeared as a doublet ($J_{\text{CC}} 44$ Hz) originating from C-6 of MVA and a signal at $\delta_{\text{C}} 20.48$ (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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