

Biosynthesis of 24-Methylsterols from [1,2-¹³C₂]Acetate; Dihydrobrassicasterol and Campesterol in Tissue Cultures of *Physalis peruviana* and Ergosterol in Yeast

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The ¹³C labelling patterns of the two methyl groups at C-25 of dihydrobrassicasterol biosynthesized from [1,2-¹³C₂]acetate differ from those of campesterol and 24-methylenecholesterol obtained from cultured cells of *Physalis peruviana* and ergosterol from yeast.

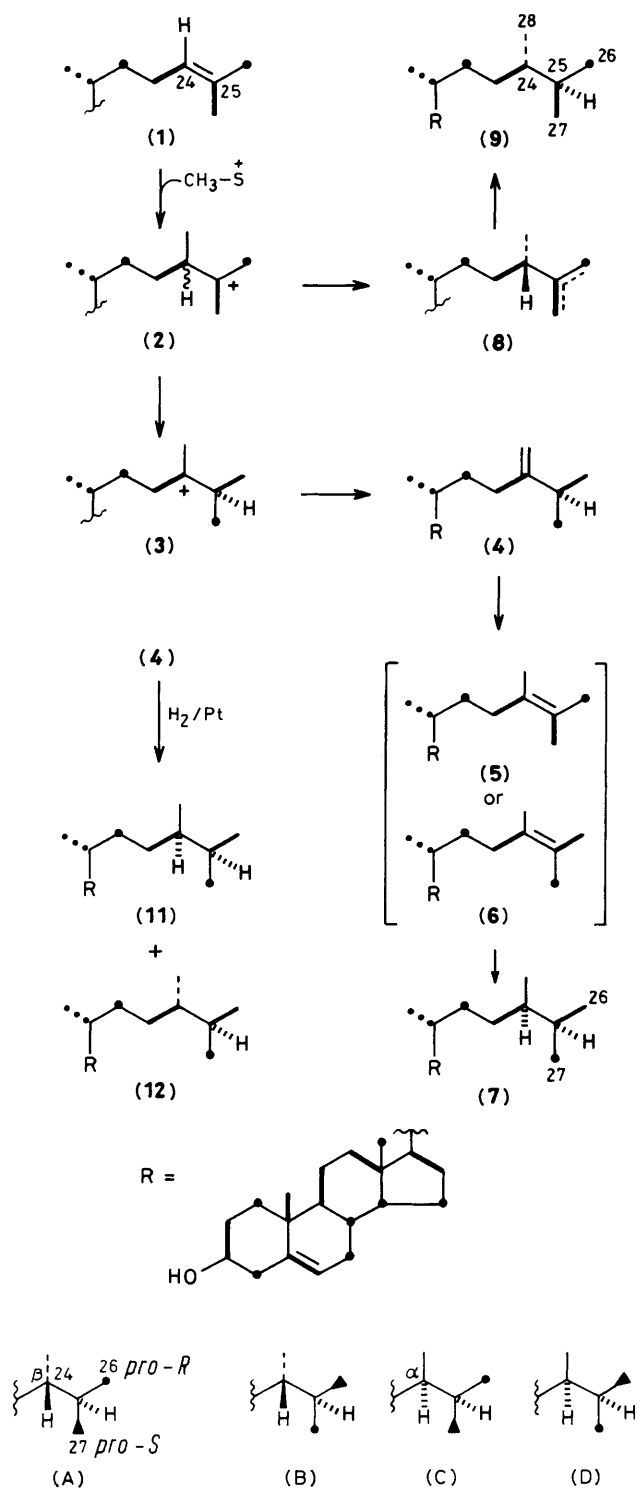
24-Methylsterols are widely distributed in nature. The 24 β -methylsterol, dihydrobrassicasterol (**9**), is frequently isolated together with its 24-epimer, campesterol (**7**), from higher

plants.¹ The biosynthetic mechanism for the formation of the 24 β -alkylsterol (**9**) has been proposed to proceed *via* hydrogenation of the $\Delta^{25(26)}$ (**8**)² or $\Delta^{23(24)}$ intermediate,³

Table 1. ¹³C N.m.r. data of sterol side-chains biosynthesized from [1,2-¹³C₂]acetate (δ_C /p.p.m. and J_{CC} /Hz in parentheses).^a

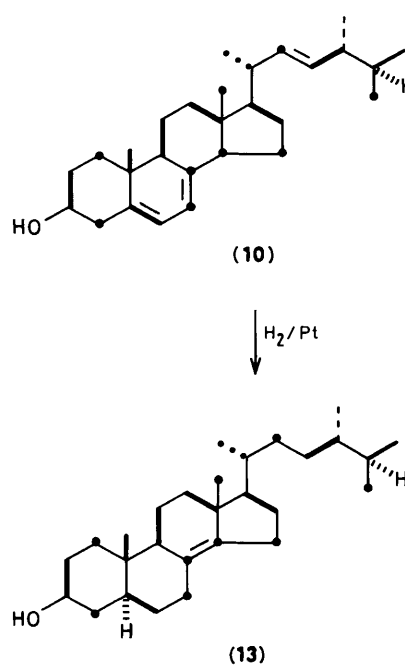
Compd.	Carbon atoms								
	C-20	C-21	C-22	C-23	C-24	C-25	C-26	C-27	C-28
(4)	35.63 (d,35)	18.65 (d,35)	34.57 (s)	30.87 (d,42)	156.26 (d,42)	33.67 (d,34)	21.92 ^b (d,34)	21.78 ^b (s)	105.56 (-)
(7)	35.75 (d,34)	18.64 (d,34)	33.60 (s)	30.18 (d,34)	38.70 (d,34)	32.30 (d,35)	20.14 ^c (d,35)	18.20 ^c (s)	15.33 (-)
(11)							20.14 ^c (d,35)	18.20 ^c (s)	
(9)	36.06 (d,35)	18.83 (d,35)	33.60 (s)	30.48 (d,34)	38.94 (d,34)	31.36 (d,35)	17.54 ^c (s)	20.45 ^c (d,35)	15.40 (-)
(12)							17.54 ^c (d,35)	20.45 ^c (s)	
(10)	40.30 (d,34)	21.06 (d,34)	135.08 (s)	131.51 (d,44)	42.68 (d,44)	32.99 (d,35)	19.91 (d,35)	19.61 (s)	17.58 (-)
(13)	34.77 (d,35)	19.26 (d,35)	33.51 (s)	30.37 (d,35)	39.07 (d,35)	31.51 (d,35)	17.62 (d,35)	20.47 (s)	15.43 (-)

^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} (in parentheses) were within 0.02 p.p.m. and 1 Hz, respectively. Multiplicities, d: doublet and s: singlet. ^b These assignments were reversed in ref. 5 and agreed with those in ref. 10. ^c These assignments are consistent with the results in ref. 11 and ref. 12.



Diastereotopic methyl groups derived from C-2 (●) or C-6 (▲) of mevalonic acid.

while the 24 α -alkylsterol (7) has been suggested to be formed via the 24-methylene derivative followed by double bond migration and hydrogenation.⁴ The two methyl groups (C-26, *pro-R* methyl group and C-27, *pro-S* methyl group) at C-25 of the sterol side-chain arise from either C-2 (●) or C-6 (▲) of mevalonic acid (MVA), as shown by (A) or (B) for 24 β -methylsterol and (C) or (D) for 24 α -methylsterol. Here we



report that the biosynthetic prochirality at C-25 of dihydrobrassicasterol (9) differs from that of campesterol (7) and 24-methylenecholesterol (4) in cultured *Physalis peruviana* cells and ergosterol (10) in yeast on the basis of the labelling patterns from [1,2-¹³C₂]acetate analysed by ¹³C n.m.r. spectroscopy.

A mixture of dihydrobrassicasterol (9) and campesterol (7) was isolated as an acetate from suspension cultures of *P. peruviana* grown in the presence of sodium [1,2-¹³C₂]acetate (180 mg/l, 1 : 2 mixture of 90 atom % enriched and unlabelled acetate). Another mixture of 24-methylsterols, (11) and (12), was prepared by chemical hydrogenation of ¹³C-labelled 24-methylenecholesterol (4), which was isolated simultaneously.⁵ The labelling patterns of C-26 and C-27 of (11) and (12) were expected to be the same as those of the starting material (4). The two specimens of the 24-methylsterol mixture obtained above were examined by ¹³C{¹H} complete decoupled and 'INADEQUATE'⁶ n.m.r. spectroscopy (τ 6.94 \times 10⁻³ s). As shown in Table 1 the two specimens of dihydrobrassicasterol (9) and (12) differ in their labelling patterns at C-26 and C-27 from [1,2-¹³C₂]acetate. A doublet signal (J_{CC} 35 Hz) was observed at δ_C 20.45 in compound (9), and at δ_C 17.54 in compound (12). For campesterol, the labelling patterns of these carbons of compounds (7) and (11) were identical, signals at δ_C 18.20 (singlet) and 20.14 (doublet, J_{CC} 35 Hz) being found. These facts indicate that dihydrobrassicasterol (9) is not biosynthesized by hydrogenation of the 24(28) double bond of 24-methylenecholesterol (4).⁷ The same labelling patterns from [1,2-¹³C₂]acetate were observed on these carbons of (9) and (7) obtained from cultured cells of *Dioscorea tokoro*.

For ¹³C signal assignments, we prepared ¹³C-labelled ergosterol (10) from [1,2-¹³C₂]acetate in yeast (*Saccharomyces cerevisiae* IFO-1346) and hydrogenated it to (24S)-24-methyl-5 α -cholest-8(14)-en-3 β -ol (13). The C-26 of this compound has been reported by Arigoni⁸ to originate from C-6 of MVA, type (B). As shown in Table 1 signals due to C-26 and C-27 appeared at δ_C 17.62 (doublet, J_{CC} 35 Hz) and 20.47 (singlet), respectively. By data comparison with compound

(13), the signals at δ_C 17.54 and 20.45 of dihydrobrassicasterol (9) were assigned to C-26 and C-27, respectively. Subsequently, based on the ^{13}C -labelling patterns, C-26 and C-27 of campesterol (7) were assigned to the signals at δ_C 20.14 and 18.20, respectively. According to the above assignments, dihydrobrassicasterol (9) has C-26 originating from C-2 of MVA, type (A) as a result of the proposed process³ (1) \rightarrow (2) \rightarrow (8) \rightarrow (9), while campesterol (7) has the same carbon derived from C-6 of MVA, type (D) after a set of proposed reactions (1) \rightarrow (2) \rightarrow (3) \rightarrow (4) \rightarrow (5) or (6) \rightarrow (7)³ in cultured cells of *P. peruviana*. It is noteworthy that 24-methylenecholesterol (4), although thought to be transformed biologically to isofucoesterol with retention of the hydrogen atom at C-25, has C-26 arising from C-6 of MVA in these cells, unlike the reported results⁹ on isofucoesterol in *Pinus pinea*. This discrepancy may reflect the existence of an unknown stereoinversion mechanism at C-25 during transformation from compound (4) to isofucoesterol.

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