Biosynthesis of 24-Methylsterols from [1,2-$^{13}C_2$]Acetate; Dihydrobrassicasterol and Campesterol in Tissue Cultures of *Physalis peruviana* and Ergosterol in Yeast

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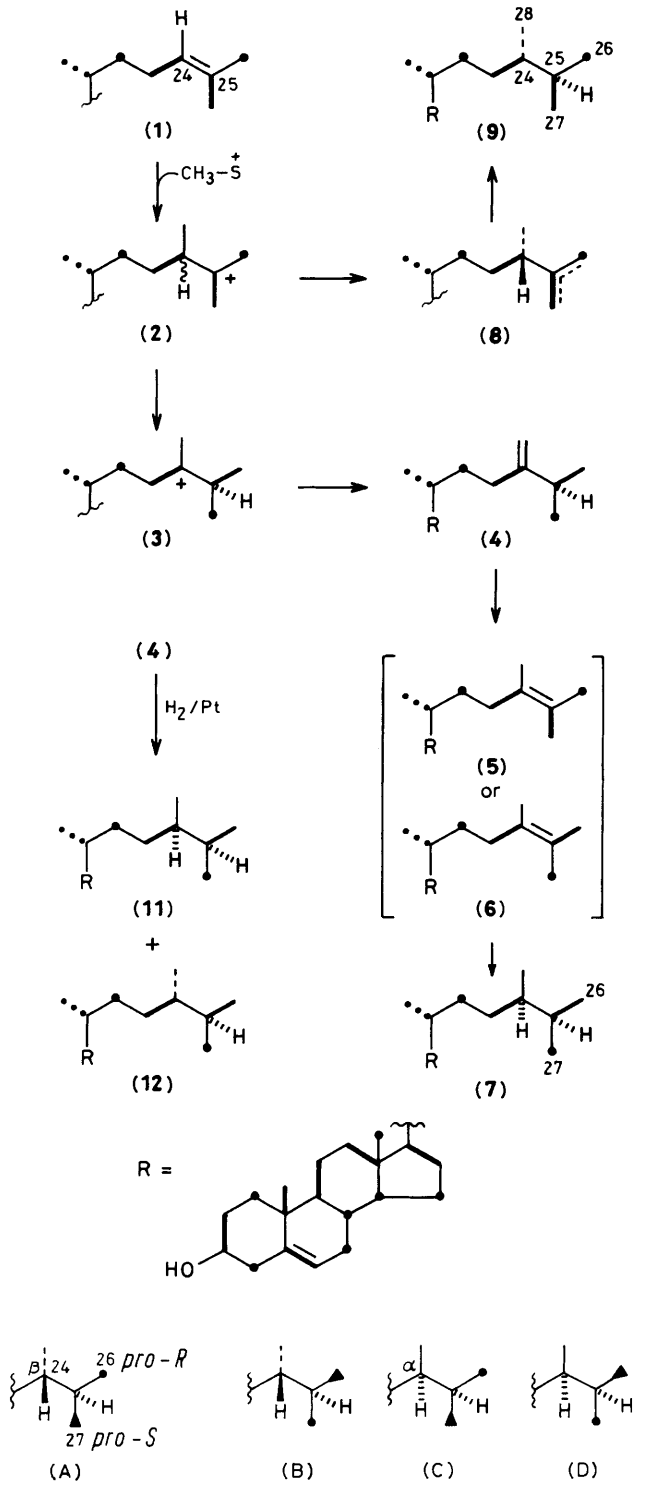
The ^{13}C labelling patterns of the two methyl groups at C-25 of dihydrobrassicasterol biosynthesized from [1,2-$^{13}C_2$]acetate differ from those of campesterol and 24-methylenecholesterol obtained from cultured cells of *Physalis peruviana* and ergosterol from yeast.

24-Methylsterols are widely distributed in nature. The 24β-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 24β-alkylsterol (**9**) has been proposed to proceed *via* hydrogenation of the $\Delta^{25(26)}$ (**8**)[2] or $\Delta^{23(24)}$ intermediate,[3]

Table 1. ^{13}C N.m.r. data of sterol side-chains biosynthesized from [1,2-$^{13}C_2$]acetate (δ_C/p.p.m. and J_{CC}/Hz in parentheses).[a]

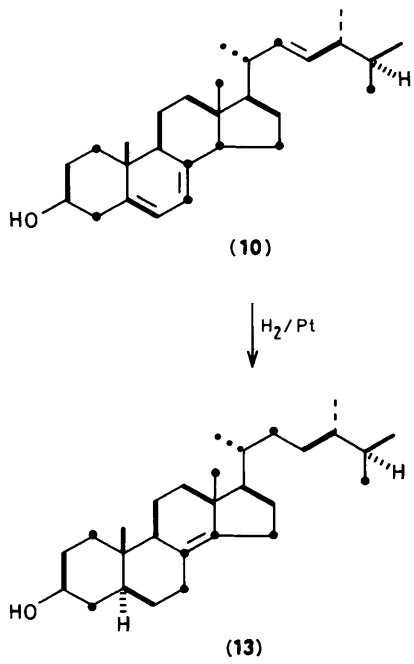
Compd.	Carbon atoms								
	C-20	C-21	C-22	C-23	C-24	C-25	C-26	C-27	C-28
(**4**)	35.63	18.65	34.57	30.87	156.26	33.67	21.92[b]	21.78[b]	105.56
	(d,35)	(d,35)	(s)	(d,42)	(d,42)	(d,34)	(d,34)	(s)	(−)
(**7**)	35.75	18.64	33.60	30.18	38.70	32.30	20.14[c]	18.20[c]	15.33
	(d,34)	(d,34)	(s)	(d,34)	(d,34)	(d,35)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

[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 Me_4Si) 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 24α-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 24β-methylsterol and (C) or (D) for 24α-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-$^{13}C_2$]acetate analysed by ^{13}C 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-$^{13}C_2$]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 ^{13}C-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 $^{13}C\{^1H\}$ complete decoupled and 'INADEQUATE'[6] n.m.r. spectroscopy (τ 6.94 × 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-$^{13}C_2$]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-$^{13}C_2$]acetate were observed on these carbons of (**9**) and (**7**) obtained from cultured cells of *Dioscorea tokoro*.

For ^{13}C signal assignments, we prepared ^{13}C-labelled ergosterol (**10**) from [1,2-$^{13}C_2$]acetate in yeast (*Saccharomyces cerevisiae* IFO-1346) and hydrogenated it to (24*S*)-24-methyl-5α-cholest-8(14)-en-3β-ol (**13**). The C-26 of this compound has been reported by Arigoni[8] 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 process[3] (**1**) → (**2**) → (**8**) → (**9**), while campesterol (**7**) has the same carbon derived from C-6 of MVA, type (D) after a set of proposed reactions (**1**) → (**2**) → (**3**) → (**4**) → (**5**) or (**6**) → (**7**)[3] in cultured cells of *P. peruviana*. It is noteworthy that 24-methylenecholesterol (**4**), although thought to be transformed biologically to isofucosterol with retention of the hydrogen atom at C-25, has C-26 arising from C-6 of MVA in these cells, unlike the reported results[9] on isofucosterol in *Pinus pinea*. This discrepancy may reflect the existence of an unknown stereoinversion mechanism at C-25 during transformation from compound (**4**) to isofucosterol.

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