Biosynthesis of (−)-β-barbatene from ^{13}C- and ^{2}H-labelled acetate, mevalonate and glycerol†

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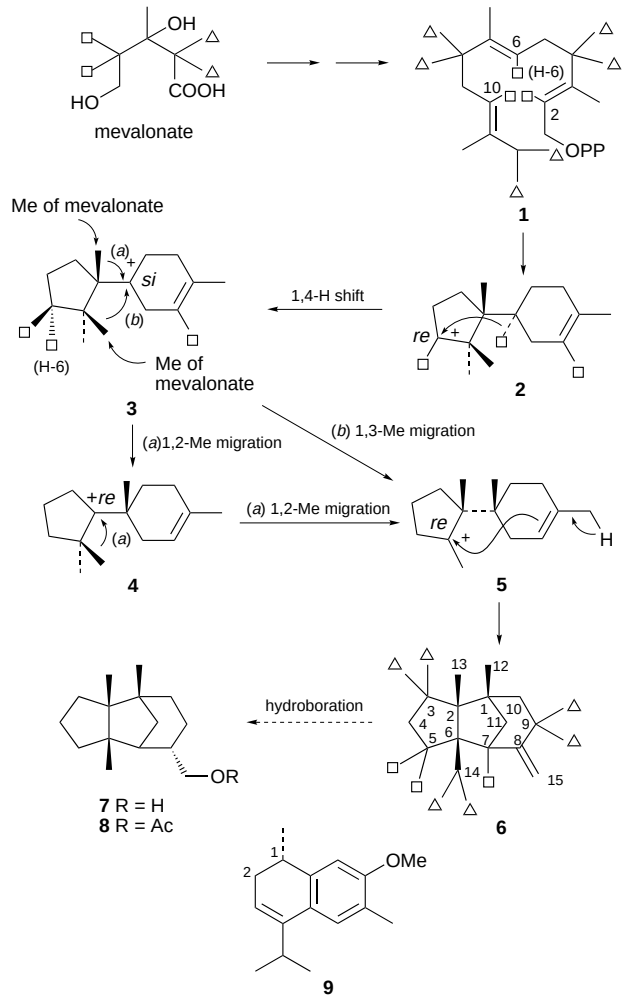
The ^{2}H and ^{13}C enrichment, ^{13}C–^{13}C coupling patterns and β-^{2}H isotope shifts observed in β-barbatanol prepared from (−)-β-barbatene incorporating variously ^{2}H- and ^{13}C-labelled mevalonates, acetates and glycerol verifies a 1,4-hydrogen shift and a double 1,2-methyl migration in the formation of β-barbatene in cultured cells of *Heteroscyphus planus*, and also indicates the diversity of regulation and the sole operation of the mevalonate pathway in extrachloroplastidic sesquiterpene biosynthesis.

The irregular sesquiterpene, β-barbatene **6**, has been proposed to be related biogenetically to the trichothecanes and cuparanes, the biosynthesis of which apparently involves a usual 1,4-hydride shift.[1] β-Barbatene is formed by further methyl migration by two routes, one involving a double 1,2-methyl migration [route (*a*) in Scheme 1], while the other features 1,3-methyl migration [route (*b*)]. Further cyclization and deprotonation of **5** affords β-barbatene. We fed deuteriated mevalonates (MVA) ([2,2-$^{2}H_2$] and [4,4-$^{2}H_2$]), ^{13}C- and ^{2}H-labelled acetates ([2-^{13}C], [1,2-$^{13}C_2$] and [2,2,2-$^{2}H_3$, 1-^{13}C]), [2-^{13}C]glycerol and [6,6-$^{2}H_2$]glucose to cultured cells of *Heteroscyphus planus* to elucidate the details of these steps and to determine whether extrachloroplastidic terpenoids are produced *via* a non-mevalonate-utilizing pathway.[2]

Cell cultures of *H. planus* were grown in MSK-4 medium[3] (75 ml), and fed 1.0 mmol of the potassium deuteriated MVAs (isotopic purity 99 atom%), 0.5 mmol of labelled acetate (isotopic purity 99 atom%), 0.5 mmol of [2-^{13}C]glycerol (60 atom%) and 11.1 mmol of [6,6-$^{2}H_2$]glucose (20 atom%) under continuous light at 25 °C. After extraction with methanol, the labelled β-barbatene was partitioned with pentane and separated by silica gel chromatography. The partially purified β-barbatene was then converted to β-barbatanol **7**, by reaction with borane–methyl sulfide, to avoid loss of volatile β-barbatene during further purification. β-Barbatanol was finally purified by repeated HPLC. Full assignment of the natural abundance ^{1}H and $^{13}C\{^{1}H\}$ NMR spectra of β-barbatanol and its acetate **8** was achieved by ^{1}H–^{1}H 2D COSY, ^{1}H–^{13}C 2D COSY, DEPT, differential NOE, DQF-COSY, PFG- HMQC and PFG-HMBC NMR studies.

$^{2}H\{^{1}H\}$ NMR spectra of β-barbatanyl [4,4-$^{2}H_2$]mevalonate indicated that ^{2}H-6 and ^{2}H-10 retained in farnesyl diphosphate **1** were incorporated into the C-5 position of β-barbatanol [δ_D 1.05 and 2.05 ($CDCl_3$), see Scheme 1] *via* the 1,4-hydride shift from cation **2** to **3** and ^{2}H-2 was incorporated into the C-7 (δ_D 1.61) position, while deuterium atoms of [2,2-$^{2}H_2$]MVA were incorporated into C-3 , C-9 and C-14 positions of β-barbatanol. ^{2}H enrichment of β-barbatanol (10.3 atom% excess) incorporating deuteriated MVA was determined by GC–MS analysis.[4] These labelling patterns indicated the 1,4-hydride shift and migration of the methyl group originating from the C-3 methyl of MVA. $^{13}C\{^{1}H\}$ NMR examination of the β-^{2}H isotope shifts[5] in β-barbatanyl [1-^{13}C, 2,2,2-$^{2}H_3$]acetate indicated the retention of two ^{2}H atoms at C-5 (ratio of $C^{2}H_2 : C^{2}H^{1}H : C^{1}H_2$ of C-4 = *ca.* 1 : 2 : 5, $\Delta\delta$ −0.22 and −0.11 ppm, Table 1) which supports the 1,4-hydride shift. No apparent ^{13}C signals due to a β-isotope shift of the C-2 carbon by $^{13}C^{2}H_3$ were observed.

The $^{13}C\{^{1}H\}$ NMR spectrum of β-barbatanyl [1,2-$^{13}C_2$]acetate showed three ^{13}C enriched peaks with doublets due to ^{13}C–^{13}C coupling (C-1–C-12, C-2–C-13 and C-8–C-15, see footnote of Table 1). The relative peak intensity of doublet to the central ^{13}C peak of C-13 (0.17) was much lower than that of C-15 (0.77) or that estimated on the basis of average ^{13}C enrichment (0.76 atom% excess), indicating that [1,2-$^{13}C_2$]acetate was not incorporated into the C-2 and C-13 positions. Intense ^{13}C–^{13}C couplings between C-1–C-10, C-4–C-5,



Scheme 1 Biosynthetic pathway of (−)-β-barbatene from deuteriated mevalonate in cultured cells of *H. planus*. Relative peak intensity of ^{2}H peaks at δ_D 1.73, 1.52 and 0.96 = 1 : 2 : 3 and that at δ_D 2.05, 1.61 and 1.05 = 1 : 1 : 1. H-6 in the carbocation **3** corresponds to H-6 in farnesyl diphosphate **1**.

Table 1 ^{13}C enrichment of carbons in β-barbatanol acetates incorporating ^{13}C- and ^{2}H-labelled acetates. Figures in parentheses show ^{13}C enrichment (atom% excess). Figures in square brackets show ^{13}C chemical shift of β-barbatanol

	δ_C					[1-^{13}C,$^{2}H_3$]Acetate incorporation	
Carbon	Non-labelled β-barbatanol	[2-^{13}C]	[1,2-$^{13}C_2$]	^{13}C–^{13}C Coupling observed[a]	[1-^{13}C,$^{2}H_3$]	$^{2}H:{}^{1}H$[b]	$\Delta\delta$[c] (ppm)
1	43.3 [43.4]	43.3 (0.8)[d]	43.3 (−0.1)	C-10, C-12 and C-11			
2	54.7 [54.7]		54.7 (0.4)	Unresolved	54.7 (2.7)		
3	34.2 [34.1]	34.2 (1.8)	34.1 (0.6)	C-2 and C-4			
4	27.9 [27.9]		27.9 (1.3)	C-5 and C-3	27.9 (0.8)	19 : 40 : 100[e]	−0.22, −0.11
5	36.7 [36.7]	36.7 (1.8)	36.7 (0.5)	C-4 and C-6			
6	54.8 [54.9]		54.8 (0.2)	Unresolved	54.8 (0.7)		
7	46.5 [46.3]	46.5 (0.8)	46.5 (0.7)	C-11, C-6 and C-8			
8	42.8 [46.8]		42.5 (0.7)	C-15, C-7 and C-9	42.5 (0.8)	30 : 32 : 100[e]	−0.18, −0.09
9	23.5 [23.4]	23.5 (1.0)	23.5 (1.2)	C-8 and C-10			
10	37.7 [37.9]		37.7 (1.3)	C-1 and C-9	37.7 (1.6)		
11	48.6 [48.7]		48.5 (0.6)	C-7 and C-1	48.5 (0.3)	31 : 100	−0.13
12	24.6 [24.7]	24.6 (0.4)[d]	24.6 (0.8)	C-1			
13	23.0 [23.0]	23.0 (1.1)	23.0 (1.2)	C-2			
14	29.0 [29.0]	29.0 (1.8)	29.0 (1.3)	C-6			
15	68.6 [67.2]	68.6 (1.8)	68.6 (0.8)	C-8			
Acetyl Me	21.1						
Acetyl C=O	171.3						
Average		1.24	0.76	1.14			

[a] Coupling constant in β-barbatanol incorporating [1,2-^{13}C]acetate, $J_{C,C}$/Hz, C-2–C-3 33.0, C-3–C-4 33.0, C-4–C-5 32.3, C-5–C-6 34.0, C-7–C-8 36.0, C-8–C-9 30.5, C-9–C-10 33.2, C-10–C-1 34.1, C-11–C-1 31.7, C-11–C-7 31.7, C-12–C-1 37.9, C-13–C-2 31.8, C-14–C-6 29.3, C-15–C-8 37.9. $J_{C\text{-}1,\,C\text{-}2}$, $J_{C\text{-}2,\,C\text{-}6}$ and $J_{C\text{-}6,\,C\text{-}7}$ were not determined, because of the low intensity of quaternary carbon atoms. [b] Ratio of carbon peak intensities for β-isotope shifted signals. [c] β-Isotope shifts due to ^{2}H. [d] Coupling constant in β-barbatanol incorporating [2-^{13}C]acetate, $J_{C,C}$/Hz, C-1–C-12 31.4. [e] $CD_2:CDH:CH_2$.

C-7–C-11 and C-8–C-15 were confirmed by a PFG-INADEQUATE experiment.[6]

Despite the low level of incorporation, the results of feeding cultured cells with [1,2-$^{13}C_2$]acetate demonstrated that all the carbon atoms in β-barbatanol were coupled with every adjacent carbon atoms. Couplings were observed between carbon atoms of different acetate units or those of different isoprene units, C-2–C-13, C-3–C-4 and C-9–C-10 (see footnote of Table 1). This suggests that β-barbatene biosynthesis is compartmentalized and occurs rapidly, *e.g.* within organelles.[7] However, in the formation of labelled (1*S*)-7-methoxy-1,2-dihydrocadalene[3] (cadinane **9**, 0.80 atom% excess) incorporating [1,2-$^{13}C_2$]acetate, which was isolated together with labelled β-barbatanol from the pentane extract of cultured cells fed with [1,2-$^{13}C_2$]acetate, no coupling was observed between the carbons of the different isoprene units. Contrasting results for β-barbatene and the cadinane suggest that their biosynthesis is regulated differently. These findings suggest a diversity of regulation in sesquiterpene biosynthesis. Although cultured cells of *H. planus* accumulate both cadinanes and bisabolanes,[3] only cadinane synthases have been detected in the 40 000 g supernatants.[8] This observation supported the suggestion that the cyclases which form bisabolanes including β-barbatene are associated with organelles, while cadinane synthases are localized in cytosol.

Labels were detected as intense singlets at C-2, C-4, C-6, C-8, C-10 and C-11 of β-barbatanol incorporating [2-^{13}C]glycerol, all of which were observed as intense singlet peaks. No deuterium atoms from [6,6-$^{2}H_2$]glucose were incorporated into β-barbatanol.

The labelling pattern supported the sole operation of the mevalonate pathway in biosynthesis of the extrachloroplastidic sesquiterpenes. In contrast the simultaneous operation of two distinct pathways, a mevalonate- and a non-mevalonate-mediated pathway, has been identified in the formation of the phytyl side-chain within chloroplasts.[9]

These obsevations are consistent with the occurrence of a 1,4-hydride shift and double 1,2-methyl migration during formation of β-barbatene and exclude the possibility of 1,3-methyl migration. They also suggested that the diversity of regulation and the sole operation of the mevalonate-utilizing pathway in the extrachloroplastidic biosynthesis of sesquiterpenes.

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Footnotes and References

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1 D. E. Cane, in *Biosynthesis of Isoprenoid Compounds*, ed. J. W. Porter and S. L. Spurgeon, Wiley, New York 1981, vol. 1, p. 283.
2 H. Rohmer, M. Knani, P. Simonin, R. Sutter and H. Sahm, *Biochem. J.*, 1993, **295**, 517.
3 K. Nabeta, K. Katayama, S. Nakagawara and K. Katoh, *Phytochemistry*, 1993, **32**, 117.
4 K. Nabeta, *Dev. Food. Sci.*, 1995, **37**, 951.
5 J. C. Vederas, *Nat. Prod. Rep.*, 1987, **4**, 277 and references cited therein.
6 H. Koshino and J. Uzawa, *Bull. Magn. Reson.*, 1995, **17**, 260.
7 C. A. West, A. F. Louis, K. A. Wickham and Y.-Y. Ren, *Recent Adv. Phytochem.*, 1990, **24**, 219.
8 K. Nabeta, K. Kigure, M. Fujita, T. Nagoya, T. Takasawa, H. Okuyama and T. Takasawa, *J. Chem. Soc., Perkin Trans. 1*, 1995, 1935; K. Nabeta, M. Fujita, K. Komuro, K. Katayama and T. Takasawa, *J. Chem. Soc., Perkin Trans 1*, 1997, 2065.
9 S. Saitoh, K. Adachi, K. Komuro and K. Nabeta, Proceedings of the 41st Symposium on the Chemistry of Terpenes, Essential Oils and Aromatics, Morioka, 1997, p. 426.

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