Pergamon

Tetrahedron Letters 40 (1999) 3803–3806

TETRAHEDRON LETTERS

Plant Terpene Biosynthesis. The Biosynthesis of Linalyl Acetate in *Mentha Citrata*

Daniel J Fowler[a], John T G Hamilton[b], Andrew J Humphrey[a] and David O'Hagan[a]*

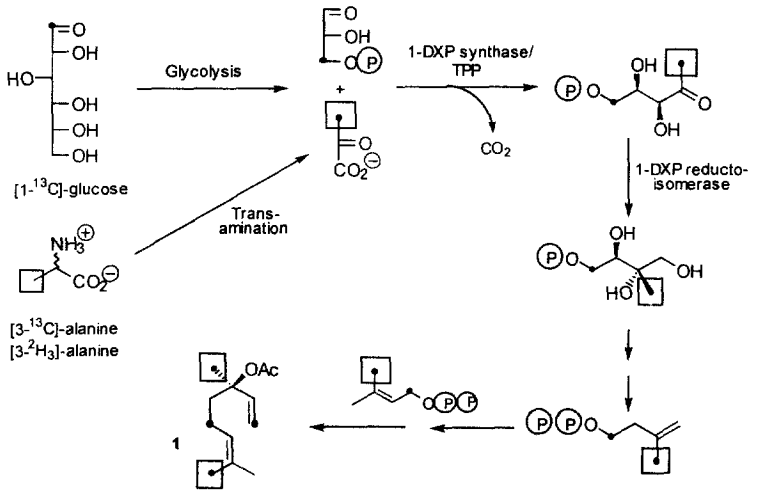
[a]Department of Chemistry, University of Durham, Science Laboratories, South Road, Durham, DH1 3LE, UK.

[b] Food Science Division, Department of Agriculture for Northern Ireland, Newforge Lane, Belfast, BT9 5PX, UK.

Received 8 February 1999; accepted 8 March 1999

Abstract: It is demonstrated that linalyl acetate is biosynthesised *via* the non-mevalonate (1-deoxyxylulose phosphate) terpene pathway in the plant *Mentha citrata*. The incorporation of isotopically labelled DL-[3-$^2H_3$]-alanine and [6,6-$^2H_2$]-D-glucose has revealed that five of the deuterium atoms of 1-deoxyxylulose phosphate are retained during the biosynthesis of linalyl acetate. © 1999 Elsevier Science Ltd. All rights reserved.

The non-mevalonate pathway, first identified in bacteria,[1] is now recognised as the pathway which delivers all of the plant monoterpenes.[2] The first intermediate on this pathway is 1-deoxyxylulose phosphate (1-DXP) which derives from the condensation of pyruvate and glyceraldehyde-3-phosphate mediated by a thiamin pyrophosphate (TPP) dependent enzyme, 1-DXP synthase. This metabolite is acted upon by a NADPH dependent reducto-isomerase to generate 2*C*-methyl-D-erythritol phosphate. A bacterial reducto-isomerase has recently been over-expressed[3] from *E. coli* and is the first enzyme to be identified which is unique to the non-mevalonate pathway. It is not clear at present how 2*C*-methyl-D-erythritol phosphate is processed in bacteria or in plants to isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP), the key precursors to terpene assembly. In order to place some limitations on the possible intermediates along the non-mevalonate pathway three papers[4,5,6] have reported the incorporation of deuterium atoms into the phytyl chain of the bacterial co-factor co-enzyme-Q. These studies revealed retention of deuterium atoms into co-enzyme-Q from positions C1, C3 and C4 of 1-DXP[4,5] and from positions C-1 and C-4 of 2*C*-methyl-D-erythritol.[6]

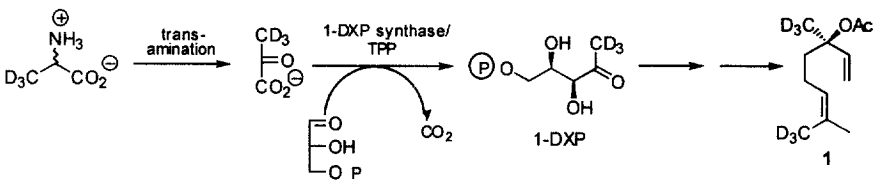


Scheme 1

PII: S0040-4039(99)00532-8

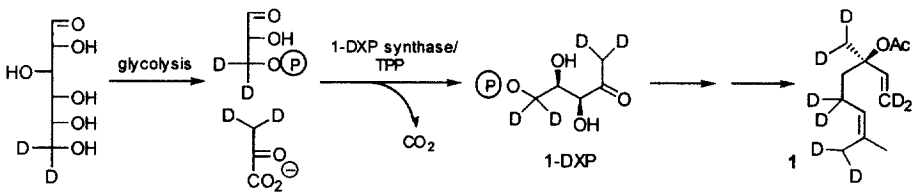
These observations are intriguing and place substantial limitations on the nature of the unknown intermediates between 2*C*-methyl-D-erythritol and IPP/DMAPP. To explore this issue further we now report deuterium incorporations into the monoterpene, linalyl acetate **1** from transformed tissue cultures of the plant *Mentha citrata*.[7] It is not clear at present if the non-mevalonate pathway operating in plants is the same in detail as that in bacteria and to this end we present studies which trace five of the hydrogen atoms of 1-DXP through the metabolic pathway during linalyl acetate **1** biosynthesis. A preliminary experiment explored the incorporation pattern in linalyl acetate from a feeding experiment[8] with [1-^{13}C]-glucose. ^{13}C-NMR Analysis revealed an isotope distribution in **1** consistent with the non-mevalonate terpene pathway. [1-^{13}C]-Glucose is processed to [3-^{13}C]-glyceraldehyde-3-phosphate as illustrated in Scheme 1 and is converted into 1-DXP by 1-DXP synthase giving rise to a measurable enhancement in the ^{13}C-NMR resonances of **1** at positions corresponding to C-1, C-5, C-8 and C-10 (see Table 1). A complementary experiment[8] with sodium [1,2-$^{13}C_2$]-acetate revealed only incorporation into the non-terpene derived acetate moiety (C-11 and C-12) of linalyl acetate **1** with no detectable ^{13}C-NMR enrichments into the carbons of the terpene skeleton. The mevalonate pathway would be expected to deliver significant label from [1,2-$^{13}C_2$]-acetate to **1**. The [1-^{13}C]-glucose result is rationalised in Scheme 1 and is consistent with previous results and expectation[1,2] for the operation of the non-mevalonate pathway.

It was pertinent to trace the fate of the hydrogen atoms of the methyl group of pyruvate and therefore the methyl group of 1-DXP, through the non-mevalonate pathway in a plant system. In a previous biosynthesis study[9] DL-alanine was successfully used as a pyruvate surrogate, and this appeared an appropriate strategy here. The isotope labelling patterns derived from ^{13}C-NMR analysis after a DL-[3-^{13}C]-alanine feeding experiment[8] labelled linalyl acetate in a regiospecific manner at two positions, C-8 and C-10 (the boxed carbons in Scheme 1 and Table 1), consistent with an intermediacy of [3-^{13}C]-pyruvate.



Scheme 2

The study was then extended to DL-[3-$^{2}H_3$]-alanine and the resultant **1** was analysed by GC-CIMS, monitoring enrichments associated with the m/z 137 fragment **B** (MH^+ -HOAc). This precursor should label 1-DXP at the C-1 methyl group as illustrated in Scheme 2. It was clear that there was a low but significant enhancement of the ion at m/z 140 of the resultant **1** as indicated in Table 2. From this experiment we conclude that all three deuterium atoms of DL-alanine (and therefore pyruvate and 1-DXP) are retained as they are processed through the non-mevalonate pathway.



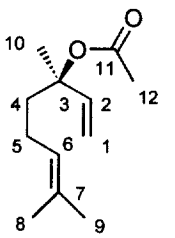
Scheme 3

In a complementary study a feeding experiment[8] with [6,6-$^{2}H_2$]-glucose was carried out. Deuterium retention should label[10] both C-1 and C-5 of 1-DXP as illustrated in Scheme 3 and be processed through to **1** labelling positions C-1, C-5 C-9 and C-10 (see Table 2). In the event there was a significant M+2 incorporation (5.96%) of two deuterium atoms into linalyl acetate, and an M+4 enrichment in ion m/z 141 (0.361%) close to the statistical (5.56 x 5.56%) level of incorporation expected for a lower population of

Carbon No.	δ/ppm[a]	[1-^{13}C]-glucose	[1,2-$^{13}C_2$]-acetate	[3-^{13}C]-alanine
1	113.3	**2.50**	-	1.10*
2	124.0	1.60	-	1.25
3	83.07	1.10*	-	1.54
4	39.88	1.40	-	1.22
5	22.54	**2.29**	-	1.50
6	142.0	1.31	-	1.28
7	132.0	1.58	-	n.d.
8	17.76	**2.31**	-	**2.00**
9	25.87	1.66	-	1.22
10	23.87	**2.44**	-	**1.90**
11	170.1	1.13	**1.68** (J_{CC} 58.9 Hz)	n.d.
12	22.36	**3.90**	**1.56** (J_{CC} 58.9 Hz)	**2.33**

[a] all samples run in $CDCl_3$ and signals referenced to $CDCl_3$ at 77.0 ppm
*signal with lowest ^{13}C enrichment referenced to 1.10% ^{13}C.

Table 1: ^{13}C NMR Derived Incorporations (%) from labelled precursors into linalyl acetate from *M. citrata*. Values in bold type indicate enhanced ^{13}C signals



Ion	[6,6-$^{2}H_2$]-glucose*	[3-$^{2}H_3$]-alanine*
137	91.692 ± 0.33	99.47 ± 0.09
138	1.844 ± 0.29	0.300 ± 0.10
139	**5.963** ± 0.19	-0.02 ± 0.05
140	0.200 ± 0.05	**0.23** ± 0.02
141	0.361 ± 0.05	0.02 ± 0.01

*GC-CIMS analysis was performed 10 times and the values represent the mean abundance relative to unlabelled linalyl acetate. GC-CIMS was carried out on a Hewlett Packard Ultra 1 column and methane was employed as the reagent gas

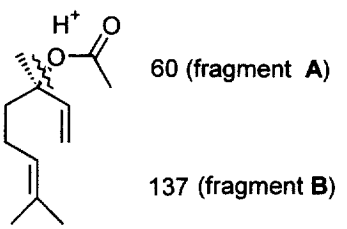


Table 2: GC-MS Derived relative isotopic abundances of fragment **B** of linalyl acetate after feeding experiments with labelled precursors. Values in bold type indicate enhanced ion abundance due to isotopic incorporation.

linalyl acetate **1** molecules carrying four deuterium atoms as a result of isotope incorporation into both C_5 units during monoterpene biosynthesis.

In conclusion five deuterium atoms of 1-DXP have been traced through the non-mevalonate pathway in plants during monoterpene biosynthesis. All five of these deuterium atoms are retained, consistent with the recent observations reported[4,5,6] on the non-mevalonate pathway operating in bacterial co-factor biosynthesis. This information places significant restrictions on the possible intermediates between 2*C*-methyl-D-erythritol and DMAPP/IPP.

Acknowledgements

We thank Dr Richard J Robins, CNRS Nantes, France, for a kind gift of the transformed cultures of *Mentha citrata,* and we thank EPSRC for a grant and a Studentship (DJF).

References and Notes

1. (a) Flesch, G.; Rohmer, M. *Eur. J. Biochem.*, **1988**, *175*, 405. (b) Rohmer, M.; Sutter, B.; Sahm, H., *J. Chem. Soc., Chem. Commun.*, **1989**, 1471. Schwender, J.; Seemann, M.; Lichtenthaler, H. K.; Rohmer, M. *Biochem. J.*, **1996**, *316*, 73.
2. Lichtenthaler, H. K.; Schwender, J.; Disch, A.; Rohmer, M. *FEBS Letts.*, **1997**, 400, 271. (b) Eisenreich, W.; Sagner, S.; Zenk, M. H.; Bacher, A. *Tetrahedron Lett.*, **1997**, 38, 3889. (c) Sanger, S.; Latzel, C.; Eisenreich, W.; Bacher, A.; Zenk, M. H. *Chem. Commun.*, **1998**, 221.
3. (a) Kuzuyama, T.; Takahashi, S.; Watanabe, H.; Seto, H. *Tetrahedron Lett.*, **1998**, *39*, 4509. (b) Kuzuyama, T.; Takahashi, S.; Watanabe, H.; Seto, H. *Proc. Natl. Acad. Sci. USA.*, **1997**, *94*, 10600.
4. Giner, J-L.; Jaun, B.; Arigoni, D. *Chem. Commun.*, **1998**, 1857.
5. Giner, J-L.; Jaun, B. *Tetrahedron Lett.*, **1998**, *39*, 8021.
6. Duvold, T.; Calf, P.; Bravo, J-M.; Rohmer, M. *Tetrahedron Lett.*, **1997**, *38*, 6181.
7. Spencer, A.; Hamill, J. D.; Rhodes, M. J. C. *Phytochemistry*, **1993**, *32*, 911.
8. [1-^{13}C]- And [6,6-$^{2}H_2$]- glucose samples were pulse fed to *M. citrata* tissue cultures on days 5, 11 and 17 to a final concentration in the medium of 8.4mM. DL-[3-^{13}C]- And [3-$^{2}H_3$]- alanine samples were similarly fed to a final concentration of 3mM and [1,2-$^{13}C_2$]-acetate was fed to a final concentration of 10mM. In all cases tissues were extracted into heptane on day 25 and the extract analysed either by ^{13}C-NMR or GC-CIMS.
9. Chesters, N. C. J. E.; O'Hagan, D. *J. Chem. Soc., Perkin Trans. 1.*, **1997**, 827.
10. An experiment with [6,6-$^{2}H_2$]-glucose revealed a similar labelling pattern in a terpene derived cyanobacterium metabolite; Proteau, P. J. *J. Nat. Prod.*, **1998**, *61*, 841 and also Proteau, P. J. *Tetrahedron Lett.*, **1998**, *39*, 9373.