Pergamon

Tetrahedron Letters 39 (1998) 521–524

TETRAHEDRON LETTERS

Biosynthesis of Sesquiterpenoid Cyclohexenone Derivatives in Mycorrhizal Barley Roots Proceeds via the Glyceraldehyde 3-phosphate/Pyruvate Pathway

Walter Maier[1], Bernd Schneider[2] and Dieter Strack*[1]

Institut für Pflanzenbiochemie, [1]Abteilung Sekundärstoffwechsel and [2]Abteilung Naturstoffchemie, Weinberg 3, D-06120 Halle (Saale), Germany

Received 8 September 1997; revised 11 November 1997; accepted 12 November 1997

Abstract: Incorporation of [1-^{13}C]- and [U-$^{13}C_6$]glucose indicates that the biosynthesis of sesquiterpenoid cyclohexenone derivatives in mycorrhizal barley roots proceeds via the glyceraldehyde 3-phosphate/pyruvate non-mevalonate pathway.

Arbuscular mycorrhizal (AM) fungus-colonized cereals and various other members of the Poaceae accumulate sesquiterpenoid cyclohexenone derivatives in their roots.[1,2] This seems to be a widespread phenomenon in the tribes Aveneae, Poeae and Triticeae.[2] The most prominent component of the cyclohexenone derivatives was found to be blumenin[1] (**1**), i.e. 9-*O*-(2'-*O*-β-glucuronosyl)-β-glucopyranoside of blumenol C[3], i.e. 6-(3-hydroxybutyl)-1,1,5-trimethyl-4-cyclohexen-3-one. The biosynthetic route leading to blumenol C is unknown. However, its biosynthesis might be related to the formation of abscisic acid (ABA) involving a dioxygenase-catalyzed cleavage of a carotenoid as a potential precursor[4] ('carotenoid pathway'). However, formation of ABA by cyclization and modification of farnesyl diphosphate might be an alternative 'non-carotenoid pathway'.

In a first attempt to elucidate the biosynthesis of blumenol C and to study the AM fungus-induced expression of the genes encoding the enzymes involved, we ran various feeding experiments using [1,2-^{13}C]acetate (Sigma-Aldrich Chemie, Deisenhofen, Germany) and [1-^{13}C]- and [U-$^{13}C_6$]glucose (Cambridge Isotope Laboratories, Andover, MA, USA) (99% ^{13}C atom excess) with mycorrhizal barley plants (*Hordeum vulgare* L. cv. Salome inoculated with *Glomus intraradices* Schenk & Smith). Determination of the degree of ^{13}C abundance in labeled positions of blumenol C should reveal the origin of its most probable biological C_5 precursor, isopentenyl diphosphate (IPP), either formed via the well known acetate/mevalonate pathway or the alternative glyceraldehyde 3-phosphate (GAP)/pyruvate pathway ('Rohmer pathway'). The latter, discovered in eubacteria[5,6] and in green algae[7,8] has now been frequently demonstrated to occur in higher plants, and most recently in the formation of chloroplast-bound isoprenoids in primary leaves of barley.[9]

In our feeding experiments, 15 pots containing 10 barley plants each were supplied with 2 g of ^{13}C-labeled compounds, i.e. 133 mg/pot, during 47 days of mycorrhiza development. Aliquots of the compounds were supplied in 20 ml per pot at days 16, 20, 23, 28, 33 and 37. Control plants were grown without feeding the labeled compounds. The plant material, AM fungus inoculation, extraction of blumenin and analytical HPLC as well as the evaluation of the degree of fungal colonization achieved (approx. 70%) is described elsewhere.[1]

* Fax: 49 (345) 55 82 106. E-mail: dstrack@ipb.uni-halle.de

0040-4039/98/$19.00
PII: S0040-4039(97)10673-6

Freshly harvested roots from 150 barley plants from both those grown with and those without the labeled compounds, were washed with water, cut into small pieces, transferred into 600 ml of aqueous methanol, treated for 1 min with an Ultra Turrax homogenizer, and allowed to stand for 30 min with continuous stirring. The extract was filtered and the residue re-extracted two times. The combined extracts were evaporated in vacuo to dryness, the residue re-dissolved in 50 ml of water and fractionated on a polyamide column (perlon CC6, 170 x 17 mm i.d.; Macherey-Nagel, Düren, Germany) using water for the elution of blumenin. The water eluate was reduced in vacuo to a small volume and subjected to preparative HPLC (System Gold; Beckman Instruments, München, Germany) equipped with a Nucleosil 100-10 C_{18} column (VarioPrep, 10 µm, 250 x 40 mm i.d.; Macherey-Nagel, Düren). Blumenin was separated at a flow rate of 10 ml min^{-1} with a linear gradient within 50 min from solvent A (1% aqueous formic acid) to 50% of solvent B (methanol) in (A and B), followed by isocratic elution for another 60 min.

NMR measurements were performed on a Bruker AVANCE DRX 500 spectrometer at 125.75 MHz (^{13}C) and 500.13 MHz (^{1}H) in methanol-d_4. The spectra were referenced to TMS. ^{13}C chemical shifts of blumenin were assigned based on ^{1}H NMR, ^{13}C NMR, ^{1}H-^{1}H COSY, HMQC and HMBC experiments. The data obtained are listed in Table 1.

Table 1. ^{13}C NMR analysis of blumenin (**1**) from mycorrhizal barley roots after feeding of ^{13}C-labeled precursors

		[1,2-^{13}C]Acetate	[1-^{13}C]Glucose	[U-$^{13}C_6$]Glucose		
	Chemical shift	^{13}C-Abundance	^{13}C-Abundance	Satellite signal	J_{C-C}	
Position	δ	% ^{13}C	% ^{13}C	% $^{13}C^{13}C$	Hz[c]	Position[d]
1	37.3	1.10[a]	1.10[a]	26.2	36.7	12
2	48.2	1.18	1.04	33.0	38.9	3
3	202.5	1.17	**1.74**[b]	28.1	38.9	2
4	125.4	1.04	0.85	9.9		
5	170.0	1.13	1.11	25.1	40.7	13
6	52.4	1.25	1.17	27.4	33.6	7
7	26.6	1.12	**1.43**	29.8	33.6	6
8	37.8	1.15	1.15	12.5		
9	75.6	1.10	1.22	34.0	39.4	10
10	19.4	1.15	**1.49**	35.0	39.4	9
11	29.1	1.22	1.21	9.1		
12	27.6	1.24	**1.55**	30.0	36.7	1
13	25.0	1.15	**1.46**	30.5	40.7	5

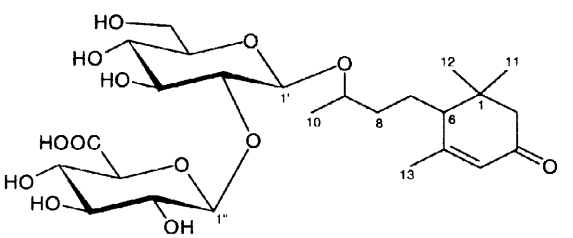
[a]The signal of C-1 was not enriched according to the control and was therefore used as reference. [b]The numbers in bold type represent significant ^{13}C incorporation from [1-^{13}C]glucose. [c]Coupling constants were determined from the ^{13}C spectrum of blumenin obtained from the experiment with [U-$^{13}C_6$]glucose. [d]Coupling partners were determined by careful inspection of the coupling constants from the ^{1}H-decoupled ^{13}C NMR and 2D INADEQUATE experiments.

^{1}H-decoupled ^{13}C NMR spectra of non-labeled (natural abundance) and ^{13}C-labeled blumenin samples (3.8 mg each in 100 µl of methanol-d_4) were measured on a 2.5 mm microprobe head under identical conditions (125.75 MHz; 27°C; 24,000 scans; acquisition time 2.26 s) using standard Bruker software. The relative ^{13}C abundance of individual carbon atoms (% ^{13}C in Table 1) was calculated as follows. The fraction of the integral of an individual ^{13}C signal of the non-labeled sample relative to the integral of the corresponding ^{13}C signal of the labeled sample were multiplied with 1.10 (natural abundance of ^{13}C).

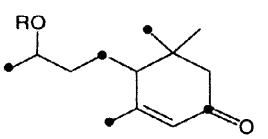
[1,2-^{13}C]Acetate was not incorporated in a significant extent into any carbon atom of the blumenin aglycone (blumenol C) nor in the disaccharide moiety. Surprisingly, under the non-sterile experimental conditions and the long incubation time, no significant scrambling of label from [1,2-^{13}C]acetate was found.

The intact incorporation of [1-^{13}C]glucose into the diglycoside part of blumenin was indicated by a ^{13}C abundance of 2.06% in C-1' and 1.81% in C-1'', while no enhancement of label was observed in other carbon atoms of the sugar moiety (data not shown). However, integrals of signals C-3, C-7, C-10, C-12, and C-13 (bold type in Table 1) of the aglycone (blumenol C) were clearly enhanced in comparison with the corresponding signal integrals of the non-labeled blumenin. The labeling pattern derived from [1-^{13}C]glucose as shown in the structure **2** is inconsistent with the acetate/mevalonate pathway but rather indicate the alternative GAP/pyruvate pathway. This conclusion is based on the well known metabolism of glucose in plants.

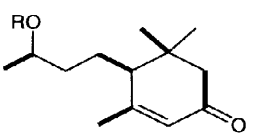
1



2



3



R = 2'-*O*-β-D-glucuronosyl-β-D-glucopyranoside

The GAP/pyruvate pathway of blumenol C has further been substantiated as follows. In the ^{1}H-decoupled ^{13}C NMR spectrum of blumenin from the [U-$^{13}C_6$]glucose feeding experiment, the fraction of multi-labeled isotopomers (% $^{13}C^{13}C$ in Table 1) was calculated as the fraction of $^{13}C^{13}C$-coupled satellites relative to the integral of the entire ^{13}C signal of the respective carbon atom. Coupling constants listed in Table 1 were determined from the satellites in that spectrum to establish the incorporation of contiguous ^{13}C-labeled isotopomers. The coupling constants accurately indicate five pairs of contiguous ^{13}C atoms. Additionally, a standard 2D INADEQUATE experiment was carried out to detect direct coupling of contiguous ^{13}C in blumenin biosynthesized from [U-^{13}C]glucose, that confirmed the occurrence of five pairs of contiguous ^{13}C-labeled isotopomers (structure **3**). Four of these pairs can be assembled into two isoprenoid moieties while C-9/C-10 might be the remainder of another isoprenoid unit of a hitherto hypothetical sesquiterpene precursor of blumenol C.

In summary, the present communication affords a further example of an isoprenoid shown to be formed via the GAP/pyruvate pathway in higher plants. Examples include isoprene[10], monoterpenoids[11], diterpenoids[12,13], as well as carotenoids, prenyl chains of chlorophylls and plastoquinone-9.[9] The pivotal intermediate in this pathway was shown to be 1-deoxy-D-xylulose[9,14], most likely as the 5-phosphorylated condensation product of GAP and pyruvate, that is converted through structural rearrangement via 2-C-methyl-D-erythritol 4-phosphate[15,16] to the branched carbon skeleton of IPP. Whether blumenol C derives directly via these intermediates from a sesquiterpene precursor or from degradation of a carotenoid structure awaits further studies.

Acknowledgements. We thank Dr. D. Gröger for helpful discussions. We also thank Gerlinde Waiblinger for skillful technical assistance. This work was supported by the Deutsche Forschungsgemeinschaft, Bonn, and the Fonds der Chemischen Industrie, Frankfurt/Main.

References

1. Maier, W.; Peipp, H.; Schmidt, J.; Wray, V.; Strack, D. *Plant Physiol.* **1995**, 109, 465-470.
2. Maier, W.; Hammer, K.; Dammann, U.; Schulz, B.; Strack, D. *Planta* **1997**, 202, 36-42.
3. Galbraith, M. N.; Horn, D. H. S. *J. Chem. Soc. Chem. Commun.* **1972**, 113-114.
4. Parry, A. D.; Horgan, R. *Physiol. Plant.* **1991**, 82, 320-326.
5. Rohmer, M.; Knani, M.; Simonin, P.; Sutter, B.; Sahm, H. *Biochem. J.* **1993**, 295, 517-524.
6. Rohmer, M.; Seemann, M.; Horbach, S.; Bringer-Meyer, S.; Sahm, H. *J. Am. Chem. Soc.* **1996**, 2564-2566.
7. Schwender, J.; Seemann, M.; Lichtenthaler, H. K.; Rohmer, M. *Biochem. J.* **1996**, 316, 73-80.
8. Schwender, J.; Lichtenthaler, H. K.; Disch, A.; Rohmer, M. In *Physiology, Biochemistry and Molecular Biology of Plant Lipids*, J. P. Williams, M. U. Khan and N. W. Lem, Kluwer Academic Publ., Dordrecht, 1997, 180-183.
9. Lichtenthaler, H. K.; Schwender, J.; Disch, A.; Rohmer, M. *FEBS Letters* **1997**, 400, 271-274.
10. Zeidler, J. G.; Lichtenthaler, H. K.; May, H. U.; Lichtenthaler, F. W. *Z. Naturforsch.* **1997**, 52c, 15-23.
11. Eisenreich, W.; Sagner, S.; Zenk, M.H.; Bacher, A. *Tetrahedron Lett.* **1997**, 38, 3889-3892.
12. Schwarz, M. K. *Ph. D. Thesis*, ETH Zürich, 1994.
13. Eisenreich, W.; Menhard, B.; Hylands, P. J.; Zenk, M. H.; Bacher, A. *Proc. Natl. Acad. Sci. USA* **1996**, 93, 6431-6436.
14. Broers, S. T. J. *Ph. D. Thesis*, ETH Zürich, 1994.
15. Duvold, T.; Bravo, J.-M.; Pale-Grosdemange, C.; Rohmer, M. *Tetrahedron Lett.* **1997**, 38, 4769-4772.
16. Arigoni, D.; Sagner, S.; Latzel, C.; Eisenreich, W.; Bacher, A.; Zenk, M. H. *Proc. Natl. Acad. Sci. USA* **1997**, 94, 10600-10605.