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Biosynthesis of the iridoid glucoside, lamalbid, in *Lamium barbatum*

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ABSTRACT

The biosynthesis of the iridoid glucoside lamalbid in *Lamium barbatum*, a plant species in the Lamiaceae, was investigated by administrating ^{13}C-labeled intermediates of MVA and MEP pathways, respectively. The results demonstrated that [3,4,5-$^{13}C_3$]1-deoxy-D-xylulose 5-phosphate could be incorporated into lamalbid, whereas the incorporation of [2-$^{13}C_1$]mevalonolactone was not observed. Based on the ^{13}C labeling pattern of lamalbid and the incorporation data, we deduce that the iridoid glucoside in *L. barbatum* is biosynthesized through the MEP pathway, whereas the classic MVA pathway is not utilized.

1. Introduction

The more than 40,000 naturally occurring isoprenoids are one of the largest groups of natural products with a great number of important biological activities. Up to date, two biosynthetic pathways for the natural isoprenoids have been found and established, namely the classic mevalonic acid pathway and the novel 2-methyl-D-erythritol 4-phosphate pathway. The MVA pathway starts from consecutive condensation of three molecules of acetyl CoA (**1**), through the key intermediate mevalonic acid (MVA, **3**) and ends at isopentenyl pyrophosphate (IPP, **8**) and dimethylallyl pyrophosphate (DMAPP, **9**, Fig. 1) which are considered as the universal building blocks for all terpenoids (Spurgeon and Porter, 1981; Bloch, 1992). The MEP pathway starts from pyruvic acid (**4**) and D-glyceraldehyde 3-phosphate (**5**), via the key intermediates 1-deoxy-D-xylulose 5-phosphate (DXP, **6**) and 2-methyl-D-erythritol 4-phosphate (MEP, **7**) and ends also at **8** and **9** (Fig. 1) (Eisenreich et al., 2004; Hunter, 2007). Experiments have shown that these two pathways exist side-by-side in higher plants, and it is generally accepted that MEP **7** is the precursor of monoterpenes, diterpenes, isoprenoid hormones, carotenoids and the side-chain of chlorophylls, tocopherols and prenylquinones (Lichtenthaler, 1999; Rohmer, 1999; Phillips et al., 2008).

Iridoid glucoside compounds are protective substances in several plant species, which show extensive biological activities such as anti-inflammatory, antitumoral-chemopreventive, hepatoprotective and healing, antibacterial, and antioxidation properties (Galvez et al., 2005). Recently, iridoid glucosides with insecticidal activity (Tzakou et al., 2007), iridoids with significant inhibition on hepatitis C virus entry in vitro (Zhang et al., 2009) and with adipocyte differentiation-inhibitory and PPARα activation activities (Bai et al., 2010) were reported. The medicinal properties of iridoid compounds have attracted significant attention and have led to intensive investigation on their biosynthesis. Early precursor administration experiments with plants being rich in this type of components with [2-^{14}C]MVA (**3**) as an upstream precursor always resulted in very low incorporations. Thus, such results did not give a clear picture of the biosynthesis of iridoid glucoside compounds (Coscia et al., 1970; Guarnaccia et al., 1974; Inouye et al., 1977a,b). Nowadays it is accepted that this type of compound is biosynthesized through 10-hydroxygeraniol (**11**), 10-oxogeranial (**12**), iridodial (**13**), iridotrial (**14**) (Fig. 2) (Uesato et al., 1986; Damtoft et al., 1992a, 1995). In the investigation of the biosynthesis of secologanin in *Catharanthus roseus* cell culture by using ^{13}C-labeled D-glucose as an upstream precursor, the researchers (Contin et al., 1998) established that the MEP pathway played a major role in its biosynthesis, whereas the MVA pathway was only involved in a minor way. Eichinger et al. (1999) defined a comparable result in their study on the biosynthesis of loganin in *Rauwolfa serpentina* cells where they concluded that the minor incorporation of mevalonate into loganin resulted from metabolite exchange between the two terpenoid pathways.

In our tracer experiments using *Lamium barbatum*, which is rich in iridoid glucosides (Yang and Gao et al., unpublished work), we have found that ^{13}C-labeled **6**, the key intermediate of the MEP

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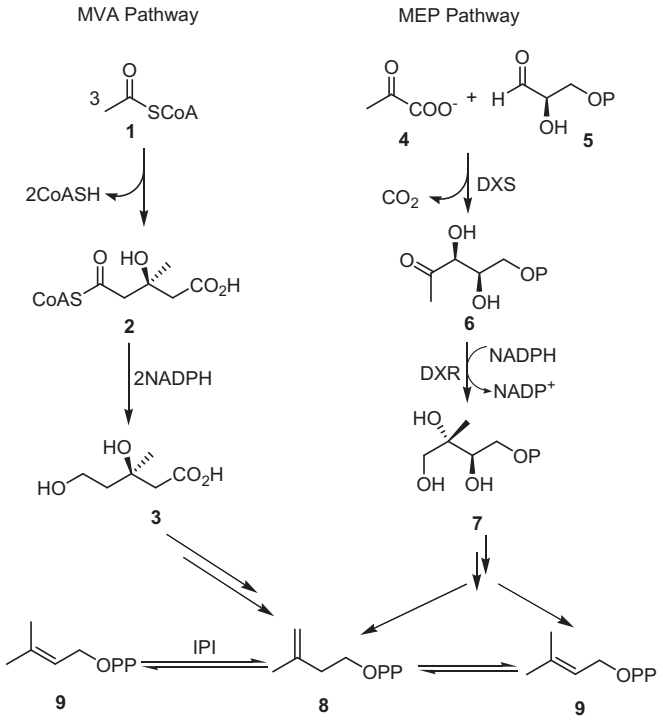

Fig. 1. MVA and MEP biosynthetic pathways for terpenoids.

pathway, is unambiguously incorporated into lamalbid (**15**, see Fig. 2), while incorporation of ^{13}C-labeled mevalonolactone (**16**), the key intermediate of the MVA pathway, was not observed. Herein, we would like to disclose our findings.

2. Results and discussion

The two isoprenoid biosynthetic pathways can each lead to specific labeling patterns for **8** and **9** when administration with ^{13}C-labeled pathway specific intermediates **6** and **16** are performed, respectively (Eisenreich et al., 2004). Accordingly, labeling in lamalbid **15** from [3,4,5-$^{13}C_3$]**6** (Fig. 2 A) and [2-^{13}C]**16** can be predicted (Fig. 2 B). If **15** is of MEP origin, the C1, C2, and C4 of both **8** and **9** should be enriched. Then head-to-tail condensation of these two building blocks should lead to the geranyl diphosphate (GPP, **10**) with positions 1, 2, 4, 6, 7, and 9 being enriched. After a few more consecutive steps of dephosphorylation, oxidation and cyclization, **15** would be enriched at C1, C2, C4, C6, C7, and C9 (Fig. 2 A). If **15** is formed, however, from MVA **3**, C4 of both **8** and **9** should possess ^{13}C label. Accordingly, lamalbid **15** with enrichment at C4 and C9 occur (Fig. 2 B).

The data obtained from the ^{13}C-labeled intermediate administration experiments and from the control are listed in Table 1. The enriched positions in **15** supported the intermediacy of DXP **6**. Moreover, the ^{13}C NMR signals of C1, C2, C4, C6, and C7 of **15** obtained from the ^{13}C-DXP administration experiment displayed satellites due to ^{13}C–^{13}C coupling (see Supplementary data). The C1 showed a doublet with a ^{13}C–^{13}C coupling constant of 44 Hz with the central resonance being from unlabeled **15**. The C4 also gave a doublet ($J_{13C-13C}$ = 40 Hz) with the alternative peak overlapping with the C3 signal. The other three carbons (C2, C6, and C7) all displayed broad triplets with ^{13}C–^{13}C coupling constants of 42, 41,

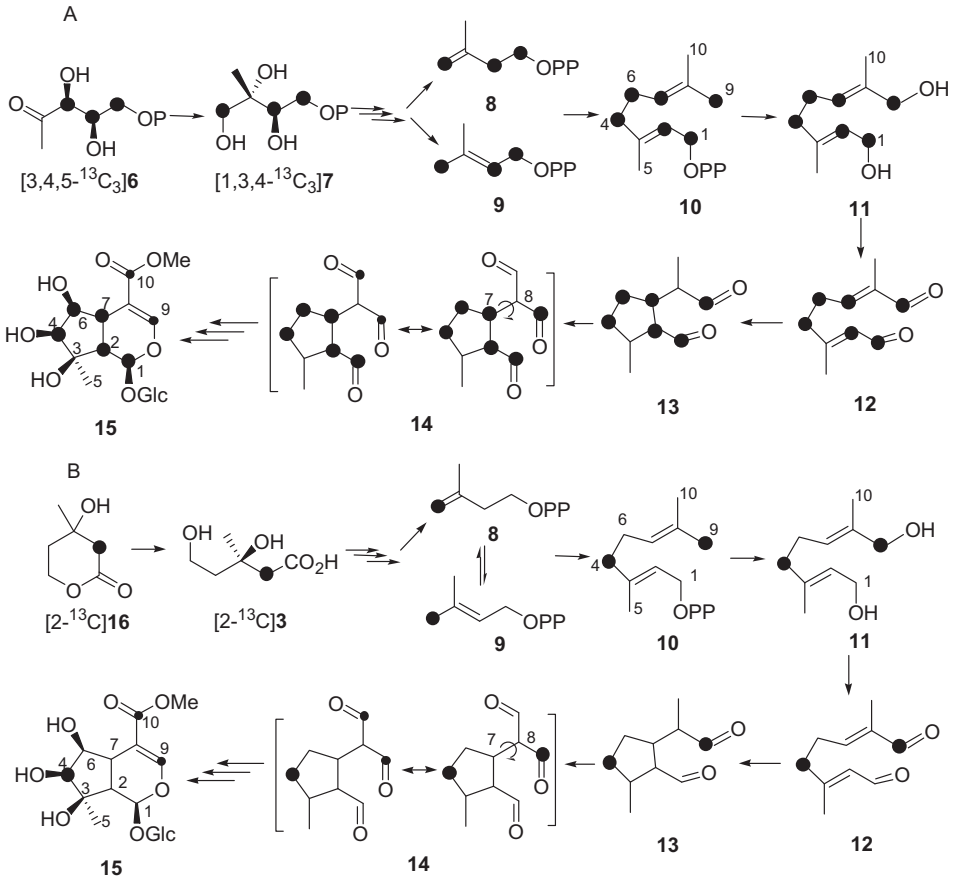

Fig. 2. The analysis of possible labeling patterns of **15** after precursor administration. Administration experiments with A: [3,4,5-$^{13}C_3$]**6** and B: [2-^{13}C]**16**.