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FORMATION OF LIRIODENDRITOL IN *LIRIODENDRON TULIPIFERA*

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Key Word Index—*Liriodendron tulipifera*; Magnoliaceae; liriodendritol; cyclitols; D-ononitol; D-bornesitol.

Abstract—Incorporation studies with tulip tree leaves, *Liriodendron tulipifera* L., using labelled inositols show that the formation of liriodendritol starts in fully expanded adult leaves and D-ononitol functions as precursor. Bornesitol contained in these leaves was identified as D-bornesitol.

INTRODUCTION

Among the multitude of *O*-methyl-inositols occurring in plants, di-*O*-methyl derivatives constitute only a minority with only three compounds identified so far. While the biogenetic precursors of dambonitol [1] and (+)-pinpollitol [2] are well known, the formation of the third, liriodendritol (1-D-1,4-di-*O*-methyl-*myo*-inositol), was subject to speculation because of the presence of two possible precursor compounds, bornesitol and ononitol, present in the leaves of the tulip tree, *Liriodendron tulipifera* L. [3]. With regard to ononitol, it was tacitly assumed that the configuration was D-(+) ononitol, since hitherto no other corresponding enantiomer had been detected in plants. In contrast, bornesitol exists in nature as D-(−) or L-(+) bornesitol [4]. On the basis of its structure only the D-(−) form would be suitable as a precursor for liriodendritol. Though the determination of biosynthetic pathways of inositol derivaties in general had been aided by the evaluation of experiments, where leaf constituents were labelled by a pulse of photosynthetic fixation of $^{14}CO_2$ followed by a chase period in $^{12}CO_2$ for a variable length of time, the corresponding experiment with tulip tree leaves gave no clear indication of a certain pathway. Therefore we had to resort to feeding labelled inositol precursors. Furthermore, the chirality of bornesitol had to be elucidated.

RESULTS AND DISCUSSION

The $^{14}CO_2$ pulse-chase experiment was carried out in spring with young, still expanding leaves of *Liriodendron tulipifera* L., since methylation and conversion of inositols in general proceeds best in very young plant tissues and ceases upon leaf maturation and subsequent aging. However, apart from *myo*-inositol, only bornesitol and ononitol became labelled, but even after a period of three weeks no processing and incorporation of label into liriodendritol had taken place. This negative result was corroborated by feeding to young tulip tree leaves [U−^{14}C] labelled *myo*-inositol, which was only converted to phospholipids, pectic compounds and both above mentioned mono-methyl inositols. Since the chirality of the bornesitol formed was unclear, this labelled compound was isolated from the chromatograms and fed via the transpiration stream to a young leaflet of *Acer pseudoplatanus*. This plant is known to specifically epimerize D-bornesitol to L-quebrachitol [5]. After an incubation time of 48 hr, 62% of the administered labelled bornesitol were found converted to L-quebrachitol. Thus it is clear that tulip tree leaves contain D-bornesitol, which in contrast to the L-configuration would theoretically be suitable as a precursor of liriodendritol.

The feeding experiments using [U−^{14}C] *myo*-inositol repeated with fully expanded leaves (Table 1) yielded the desired labelling of liriodendritol of 8.4% after 72 hr; however, no clear decision could be made between D-ononitol and D-bornesitol with respect to a precursor function. The comparatively high rate of label incorporation into D-ononitol or the low rate of D-bornesitol formation cannot be interpreted unequivocally. Therefore [U−^{14}C] labelled D-ononitol and D-bornesitol were fed to adult tulip tree leaves. While D-bornesitol was only demethylated to a minor degree to yield *myo*-inositol, D-ononitol was converted by 15.8% into liriodendritol. The latter experiment was repeated with only D-ononitol as substrate in October, shortly before the leaves started to turn yellow, but then less than 1% of label was recovered from liriodendritol.

The lack of liriodendritol formation in young leaves of the tulip tree, evaluated together with the low rates of *myo*-inositol conversion with simultaneous synthesis of liriodendritol in fully expanded summer leaves, suggest a certain biosynthetic program: in spring and early summer D-ononitol (plus D-bornesitol) is being formed and possibly accumulated until the synthesis of liriodendron starts in the older, fully expanded summer leaves, utilizing the accumulated precursor. Since even the direct feeding of D-ononitol resulted in a significant but nevertheless low rate of liriodendritol formation as compared to the epimerization of the same precursor to D-pinitol in *Trifolium incarnatum* [6], we may have either missed the peak period of its synthesis or this double methylated inositol indeed is characterized by a low rate of formation. According to Angyal and Bender [3], autumn leaves of the Tulip tree contain nearly twice as much liriodendritol as D-ononitol. In contrast to L-quebrachitol, which in different species of *Acer* is translocated from the leaves

Table 1. Metabolites of two feeding experiments with fully expanded leaves of *Liriodendron tulipifera* using labelled precursors

	Feeding time (hr)				
	myo-Inositol			D-Ononitol	
Metabolites	24	48	72	24	72
myo-Inositol	83.1	78.4	62.3	0	0
D-Bornesitol	4.3	2.3	4.1	0	0
D-Ononitol	12.6	15.5	23.3	90.7	84.2
Liriodendritol	0	2.6	8.4	9.3	15.8
Unidentified	0	1.2	1.9	0	0

Data given in % of total radioactivity recovered from the leaf tissue. Compounds fed were uniformly labelled with ^{14}C.

into the stem [7] and thus constitutes a major component of maple syrup [8], liriodendritol apparently is shed with the leaves, since we were not able to detect any in the cortex or wood tissue of the tulip tree.

EXPERIMENTAL

PC, radioautography and high-voltage electrophoresis for analysis and compound identification were carried out as previously described [9].

Feeding experiment. The petiole of a *Liriodendron tulipifera* leaf was shortened to 15 mm and the leaf area trimmed to a square of 15 mm side length. Placed into a small test-tube containing the aq. soln of either 10 μCi *myo*-[U−^{14}C] inositol, 1 μCi D-[U−^{14}C] ononitol or 0.5 μCi D-[U−^{14}C] bornesitol, the leaf was illuminated and slightly aerated to facilitate the uptake of the soln. Distilled H_2O was further added when the soln had been consumed and leaf sections were cut out at intervals to be analysed for labelled products. *Myo*-[U−^{14}C]inositol was fed likewise to *Trifolium incarnatum* for 14 hr and to *Acer pseudo-platanus* for 12 hr to provide labelled D-ononitol and D-bornesitol, respectively. Bornesitol (0.1 μCi) was isolated from the chromatograms of *Liriodendron tulipifera* leaves of the above described feeding expt with labelled *myo*-inositol and was likewise fed to a young, untrimmed leaf of about 30 mm^2 area of *Acer pseudo-platanus*. Only one sample was taken after 48 hr of incubation and analysed for the appearance of L-quebrachitol.

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