Lignans and lignan glucosides in stems of *Ginkgo biloba* L.

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Abstract

Outline of this thesis

This thesis is entitled "Lignans and lignan glucosides in stems of *Ginkgo biloba* L.," and it consists of five chapters.

This study aimed to investigate the lignan, lignan glucosides and neolignan composition and visualize them in stems of ginkgo by cryo-TOF-SIMS/SEM.

In chapter 1, Introduction is shown.

In chapter 2, investigation of lignan and lignan mono/diglucosides in ginkgo stem is presented. Which includes quantification and visualization of them within ginkgo stem, and proposed biosynthesis pathways of main lignan and lignan glucosides was also discussed.

In chapter 3, investigation of neolignans in ginkgo stem is presented. Which includes quantification and visualization of them within ginkgo stem, and proposed biosynthesis pathways of two structural isomers was also discussed.

In chapter 4, other substance isolated from ginkgo bark is presented. Some of them were visualized by cryo-TOF-SIMS/SEM successfully.

In chapter 5, the results and discussion of this thesis are summarized.

Chapter 1. Introduction

Lignans and neolignans are a large group of naturally occurring phenolic compounds characterized by the coupling of two C_6-C_3 units. If the two C_6-C_3 units are linked by a bond between positions 8 and 8′, the compound is named lignan; compounds with all other types of linkages are called neolignans. They differ from lignin by not having properties associated with biopolymers; they have roles in fighting phytopathogenic organisms, protecting against stress and regulating growth. They are important components in foods and medicines. Many lignans have demonstrated wide-

ranging pharmacological activity, such as honokiol and aryltetralin as anticancer lignans. They can be found either in free phenolic form or glucoside form with a large variety of various carbohydrates by glycosylation. Glycosylation is a key mechanism that ensures their water solubility, low toxicity, and stability by protecting the reactive phenolic hydroxyl groups of aglycons and is assumed to assist lignan accumulation and transportation in plant cells. Due to the complex investigation of distinct lignan and neolignan glycosides and widely related derivatives, the amounts of lignans and neolignans in various plants and tissues remain unclear.

The structural patterns of lignans, such as their carbon skeletons, the way oxygen is absorbed into the skeletons, and the cyclization pattern, are used to divide them into ten categories. They are furofuran, furan, dibenzylbutane, dibenzylbutyrolacton, dibenzylbutyrolactol, aryldihydronaphtalen, arylnaphthalene, aryltetralin, dibenzocyclooctadiene types. Neolignans are divided into fifteen subtypes, none of which have been given particular names.

The biosynthesis of lignan has been well-studied in recent years. Lignan biosynthesis starts with the coupling of two molecules of coniferyl alcohol and results in the formation of pinoresinol (furofuran). Pinoresinol is further converted to secoisolariciresinol by pinoresinol/lariciresinol reductase (PLR) via lariciresinol (furan). This biosynthetic pathway is common to several plant species. In addition, neolignan biosynthesis is also considered as starts with the coupling of two molecules of coniferyl alcohol and results in the formation of such as 8-5′, 8-O-4′-linked neolignan formation has been reported.

Chapter 2. Investigation of lignan and lignan mono/diglucosides in ginkgo stem.

Four lignans and four lignan glucosides were successfully characterized in this research. Quantitative HPLC measurements were conducted on serial tangential sections of freeze-fixed ginkgo stem to determine the amount and approximate distribution of lignan and lignan glucosides. The structures of separated lignans and lignan mono/diglucosides were confirmed by NMR, ESI MS and optical rotation. (−)-Olivil 4,4′-di-*O*-β-D-glucopyranoside (olivil DG) was the most abundant lignan glucoside in ginkgo by HPLC quantification. The secondary ion of olivil DG distribution was correspond to the HPLC results. The distributed mainly in the phloem, ray parenchyma cells, and pith.

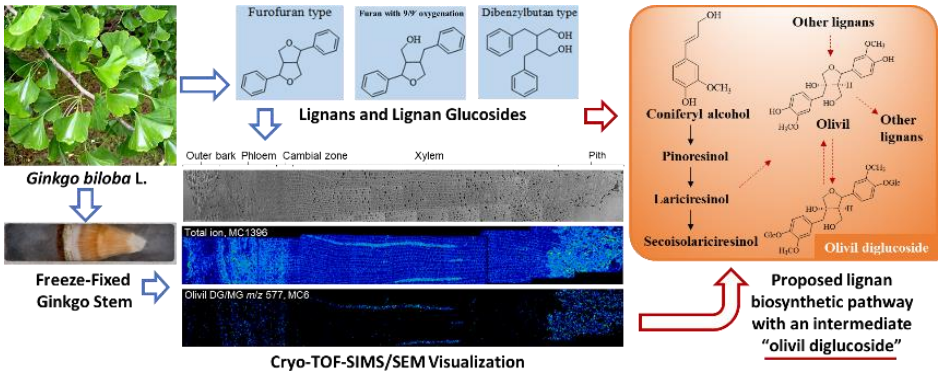

Figure 1. Schematic diagram for chapter 2

Consequently, our results revealed that olivil DG was localized not only in the parenchyma cells in the phloem, but also in the ray parenchyma cells in the xylem and parenchyma cells of the young pith. Coniferin and glycosylation lignan fill the living parenchyma cell lumens, imparting defense against pathogens by preventing their lateral and axial spread, and by accumulating lignan glucosides.

Only the actual storage locations of the target compounds could be visualized via TOF-SIMS. Therefore, the question of where they are synthesized remains unclear. In plants, biomolecules can be synthesized or derivatized, transferred, stored, and used at different positions, and it is difficult to visualize all stages of these biomolecular lives together. Nevertheless, the chemical interrelation of the lignan families with the structures, storage amount, and storage positions revealed was also discussed in this thesis.

The comparative accumulation of olivil DG revealed its possible transport pathways and storage sites in ginkgo. From the viewpoint of the chemical structure, olivil can be transformed into other lignan structures, such as ginkool and cycloolivil, which are detectable in ginkgo. Lignans can be found as different aglycones and with a variety of mono/glucosides in different binding patterns. The aglycone content was much lower than that of their glucoside form. Olivil DG might be an important intermediate and storage form in the lignan biosynthetic pathway of ginkgo.

Chapter 3. Investigation of neolignans in ginkgo stem.

To investigate the composition and distribution of neolignans in ginkgo, 2 benzofuran neolignans were isolated together from the bark of *Ginkgo biloba* L. for the first time. Their structures were elucidated by comprehensive spectroscopic data analyses. They are structural isomers of $C_{20}H_{20}O_6$ and differ from normal neolignans found in ginkgo by reported literatures. Quantification of them in ginkgo seed and stem were conducted, and surprised to find that they were prominently present in ginkgo stem extracts, but basically not exist in ginkgo seed. Coniferin was abundant in ginkgo seed and ginkgo stem aqueous extract. The actual distribution of benzofuran neolignans in flash-frozen stems of ginkgo was visualized by cryo-TOF-SIMS/SEM. Visualized distribution and quantitative analysis of this metabolism in ginkgo may prove its importance in biosynthesis of lignan and neolignan.

XH-14 and 2-(4-hydroxy-3-methoxyphenyl)-5-(3-hydroxypropyl)-6-methoxybenzofuran-3-carbaldehyde successfully isolated together from ginkgo bark. The only difference between these two compounds was the replacement of the methyl signal location. Their total content in ginkgo stem were confirmed by HPLC, and their radial distribution successfully visualized first time in ginkgo stem by cryo-TOF-SIMS/SEM. Combining previous studies, the results of this chapter revealed that coniferin was found in large amount in various parts of ginkgo, and lignan glucosides and neolignans were found in large amount in ginkgo stem.

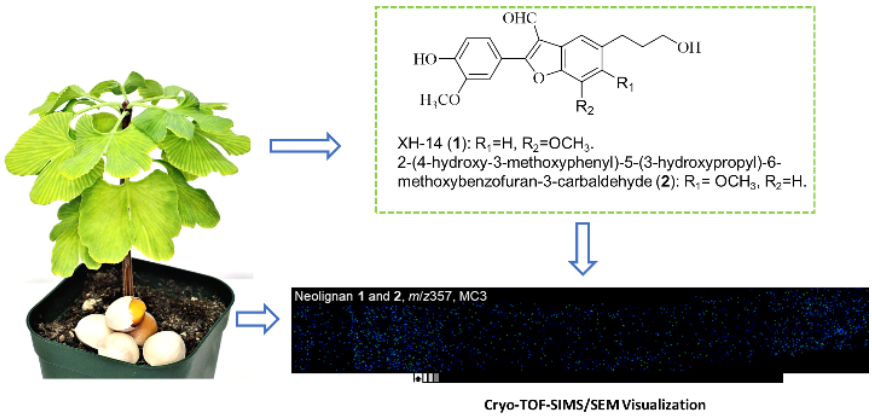

Figure 2. Schematic diagram for chapter 3

In addition, benzofuran neolignans were the most amount neolignans in ginkgo and accumulated largely in ginkgo stem, especially in parenchyma cells. Aldehyde coupled in lignan seems not normal, but it's a substrate for lignin biosynthesis in ginkgo. Coniferyl aldehyde is also the precursor of coniferyl alcohol, they are both involved in lignin monomer formation by cinnamyl alcohol dehydrogenases. The most interesting part in this study is benzofuran neolignan **2** is a highly unstable structure, which methoxy group at C-2′ lignan is rare and does not common in nature occurring, it's a free form accumulated largely in ginkgo stem. So, the comparative accumulation and novel structure of 2-(4-hydroxy-3-methoxyphenyl)-5-(3-hydroxypropyl)-6-methoxybenzofuran-3-carbaldehyde, revealed its alternative biosynthetic pathways, which are different from the formation of 8-8′ coupling lignans.

Chapter 4. Other substance isolated from ginkgo bark.

The main targets in this research are the lignan structure type substances. During the isolation and purification process, some other type compounds were also obtained together with the targets. For example, ginkgolide C, protocatechuic acid, glycerol, etc. Some of them were also characterized by NMR spectrum and visualized by cryo-TOF-SIMS/SEM.

Chapter 5. Conclusion

Lignans, lignan mono/diglucosides and neolignans were isolated, quantified and successfully visualized by cryo-TOF-SIMS/SEM from ginkgo. The results suggested that (−)-Olivil 4,4′-di-*O*-β-D-glucopyranoside is the most amount lignan in ginkgo, and might be an important intermediate and storage form in the lignan biosynthetic pathway of ginkgo. Benzofuran neolignans are the most amount neolignans in ginkgo, but not include ginkgo seed. Their biosynthetic pathway may differ from lignans. They maybe these being generated by oxidative coupling of coniferyl aldehyde alone, or coniferyl alcohol alone, or a mixture of coniferyl aldehyde and coniferyl alcohol.