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主　論　文　の　要　旨

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

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論　文　内　容　の　要　旨

To investigate the biosynthetic pathways and regulatory mechanisms of lignans in plants, the actual distributions of lignans and lignan glucosides in flash-frozen stems of *Ginkgo biloba* L. (Ginkgoaceae) were studied using cryo-TOF-SIMS/SEM. Lignans and neolignans are a large group of naturally occurring phenolic compounds. They are differing from lignin in several ways. They are important components of foods and medicines. They can be found either in free phenolic form or glucoside form with a large variety of various carbohydrates by glycosylation. Due to the complex investigation of distinct lignan glycosides and widely related derivatives, the amounts of lignans in various plants and tissues remain unclear.

In this thesis, several lignans, lignan mono/diglucosides and neolignans were extracted and purified from ginkgo. The visual distributions of the lignan glucosides, free lignans and neolignans within ginkgo stems were investigated. Direct visualization of lignan and lignan glycosides is an important step towards a better understanding of the transportation and defense functions of phenylpropanoid secondary metabolites in ginkgo. This thesis includes the first time to present the distribution of lignan, lignan glucosides and neolignans by cryo-TOF-SIMS/SEM. Their possible transport pathways were also proposed. These results provide valuable information for the exploration of lignans in pharmaceutical, cosmetic and food applications.

Investigation of lignans and lignan mono/diglucosides in ginkgo stem:

Four lignans and four lignan glucosides were successfully characterized. 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 mo/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.

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.

Investigation of neolignans in ginkgo stem:

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, including UV, ESI MS, NMR and circular dichroism (CD) spectra. They are structural isomers of $C_{20}H_{20}O_6$ and differs from normal neolignans found in ginkgo. We tried to quantify them in ginkgo stem and ginkgo seed and were surprised to find that they were more prominently present in ginkgo stem extracts. Their actual distributions in quick-frozen ginkgo stem were studied using cryo-TOF-SIMS/SEM. Their total content is about 0.69 mM per gram dry weight of ginkgo stem. The secondary ions derived from these two benzofuran neolignans was detected from phloem to pith.

From the proposed biosynthetic pathway of benzofuran neolignans, benzofuran neolignans are mostly like produced through the oxidative coupling of a combination of coniferyl aldehyde and coniferyl alcohol or alternative biosynthetic pathways, which are different from the formation of 8-8′coupling lignans.

In conclusion, lignans, lignan mono/diglucosides and neolignans were isolated, quantified and successfully visualized by cryo-TOF-SIMS/SEM from ginkgo. (−)-Olivil 4,4′-di-*O*-β-D-glucopyranoside is the most amount lignan in ginkgo, the aglycone of olivil DG might be an important intermediate and olivil DG might be the storage form in lignan biosynthetic pathway of ginkgo. Benzofuran neolignans are the most amount neolignans in ginkgo, 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.