学位論文内容の要旨

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学位論文題名

Introducing stable isotopes into small molecule to elucidate functional analysis of biologically active compounds

（生理活性物質の機能解明を目指した低分子安定同位体標識法の検討）

Isotopic labeling is a powerful technique extensively used in many aspects such as pharmaceutical industry. This technique involves incorporating either stable isotopes, such as deuterium (D) and carbon-13 (^{13}C), or radioactive isotopes, such as tritium (T) and carbon-14 (^{14}C), into molecules. By tracking these labeled molecules, researchers gain unique and invaluable insights into the pharmacokinetics and pharmacodynamics of new drug candidates. There are two main methods to achieve stable isotopic labeling into bioactive molecules, chemical labeling and metabolic labeling.

In this research, both two methods were performed, and isotopic labeling compounds were successfully obtained with high yield and effects. Deuterium-labeled indole-3-propionoic acid (IPA) was achieved through chemical synthesis under mild reaction conditions and ^{13}C-labeled squalene was obtained through metabolic biosynthesis in filamentous fungus *Trichoderma.virens* PS1-7, which can be used to elucidate the metabolic pathways of indole derivatives and steroidal substances in living organisms individually.

1. Chemical synthesis of deuterium-labeled indole-3-propionoic acid (IPA) through H/D exchange with deuterated trifluoromethanesulfonic acid (TfOD)

Indole-3-propionic acid (IPA), a member of the plant auxin family, plays an important role in biological activity. Deuterium incorporation via H/D exchange is one of the simplest ways to prepare deuterated compounds, however, there are few reports for the preparation of their deuterated derivatives for IPA. In this research, H/D exchange with deuterated trifluoromethanesulfonic acid (TfOD), can achieve our aim easily. The reactions can be conducted in a temperature-dependent manner according to the chemical stability of the ligands without the use of special reagents. The detailed analysis of H/D exchange of IPA revealed that the reaction proceeded swiftly at low

temperature. The effective H/D exchange of indole-3-alkanoic acids will be helpful not only in plant regulation studies but also in drug development.

2. Metabolic biosynthesis of ^{13}C-enriched squalene in in filamentous fungi *Trichoderma virens* PS1-7 in the presence of butenafine hydrochloride, squalene epoxidase inhibitor.

Squalene is a primary metabolite of almost all triterpenoids with polyene structure and has been attracting attention from the pharmaceutical and cosmetics industries. Filamentous fungi have biosynthesis of various terpenoids via mevalonate pathway, but few accumulations of squalene were observed under normal cell growth culture conditions. I investigated the accumulation of squalene in filamentous fungi by inhibiting squalene epoxidase, which is the first enzyme in squalene metabolism. Squalene epoxidase is essential enzymes for fungi homeostasis to biosynthesis of important steroids (ex. ergosterol). Moderate inhibition of the enzyme is required to maintain both cell growth and squalene accumulation. It is optimized that filamentous fungi, *Trichoderma virens* PS1-7, produced squalene up to 429.93 ± 51.60 mg/L after culturing for 7 days in the medium consisted of potato infusion with glucose at pH 4.0, in the presence of 200 μM butenafine hydrochloride. I conducted other eight *Trichoderma virens* strains, which are available from NBRC, because *Trichoderma virens* PS1-7 is housekeeping fungi. All *Trichoderma virens* strains were found to be suitable for squalene accumulation. I found that PS1-7 is one of the most suitable strains for squalene accumulation among *Trichoderma virens*. The results indicated that filamentous fungi *Trichoderma virens* PS1-7 can be a potential source of producing squalene with the presence of squalene epoxidase inhibitor, butenafine hydrochloride, which heavily upgraded squalene accumulation than that without butenafine. Through further optimizing cultivation conditions, the squalene can be accumulated up to 608.94 ± 46.87 mg/L in PS1-7 by culturing up to 28 days. Based on that result, [U-$^{13}C_6$]-glucose was utilized to biosynthesize [U-^{13}C]-labeled squalene in the presence of exogeneous butenafine. The biosynthesized squalene, which is C30 triterpene, contained up to 30 atoms of stable isotope ^{13}C from mass spectrometry. The high incorporation of ^{13}C enables to measure ^{13}C-^{13}C COSY, which is not easy to measure in natural product, to elucidate structural analysis of labeled squalene. The accumulation of stable isotope squalene in filamentous fungi can be utilized to analyze terpene metabolites derived from squalene.