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Growth and Isoprenoid Metabolism of Cultured *Picrasma quassioides* Cells

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Quassinoids are the specific bitter principle of Simaroubaceae plants, and are of certain interest because of their characteristic biological (e. g. antileukemic) activities[1,2]. They are believed to be biosynthesized through triterpenoid pathway[1], but the exact nature of biosynthetic intermediates (supposed to be tirucallane or euphane derivatives) and the mechanism of picrasane skeleton formation are not known. Although the production of quassioids in tissue cultures of *Picrasma quassinoides*[3] and *Ailanthus altissima*[4,5] has been reported, the yields have been very low[4] or variable[5], and no studies toward the regulation of quassinoid biosynthesis have been carried out.

One of the candidates for regulatory points in quassinoid biosynthesis is the enzymatic transformation of squalene-2, 3-epoxide into cyclic triterpenes (triterpen-3-ols)***, because this step leads to numerous types of triterpenoid skeletons including an intermediate of phytosterol biosynthesis (cycloartenol).

While *in vitro* examination of this cyclization is often hampered by experimental difficulties (e. g. nonavailability of labeled substrates and standard samples of expected products), *in vivo* incorporation experiments with radio-labeled precursors would provide useful information about the control of these branching pathways in plant cells. This communication describes such an approach with cultured *Picrasma quassioides* cells using ^{14}C-labeled mevalonic acid.

Suspension cultures of *P. quassioides* were initiated from 4-week-old callus cultures (dark, Murashige-Skoog's medium supplemented with 1 ppm 2, 4-D, 0. 1 ppm kinetin and 0. 7% coconut water). The cells did not exhibit bitter taste, and indeed no quassinoids could be detected by TLC and HPLC in the EtOAc extracts. Aliquots (20 m*l*) of the suspension cultures (250 m*l*) were transferred into other culture vessels every 7 days, and incubated with (*RS*)-[2-^{14}C] mevalonic acid

*** In this communication, for simplicity and accuracy, both cyclic triterpen-3-ols with a molecular formula of $C_{30}H_{50}O$ (including cycloartenol) and 24-methylenecycloartanol are referred to as "triterpenes"; the term "phytosterols" indicates only 4, 4-bis(demethyl)-type compounds, and excludes 4-methylsterols or 4, 4-dimethylsterols.

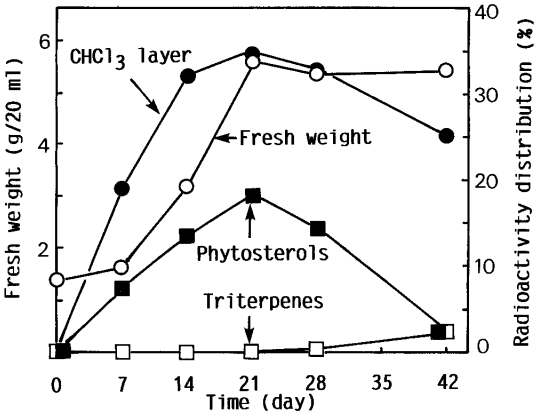

Fig. 1. Growth of suspension-cultured *Picrasma quassioides* cells (○) and biosynthesis of isoprenoids from [2-^{14}C] mevalonic acid. The cells were incubated with the radio-labeled precursor for 48 h, and radioactive products in $CHCl_3$ extracts were separated on TLC. The percentage of radioactivity recovered in total $CHCl_3$ layers (●), in phytosterol fractions (■) and triterpene fractions (□) to administered activity (185 kBq) is shown.

(185 kBq) for 48 h under the same conditions. Radioactive metabolites were extracted from the cells and analyzed with essentially the same method described previously[6]. Briefly, the $CHCl_3$ extracts were applied to silica gel TLC (solvent: toluene/EtOAc=4/1) and, after examination with radio-chromatoscanner and/or autoradiography, phytosterol (Rf=ca. 0. 35) and triterpene (Rf=ca. 0. 50) areas were scraped from TLC plates. The radioactivity was measured with a liquid scintillation counter.

The results are summarized in **Fig. 1**. The fresh weight of the suspension-cultured cells increased 4-fold in 3 weeks, and remained unchanged thereafter. Uptake of (*RS*)-[2-^{14}C] mevalonic acid from the medium into the cells was very low (ca. 3%) at the beginning of the culture (day 0), but it increased rapidly as the cells proliferated; 80-90% of the administered mevalonic acid were taken up into 2- to 4-week-old cells. A maximum of 35% of the administered radioactivity was recovered in chloroform-soluble materials. Because half (3*S*-form) of the administered mevalonic acid is not utilized for isoprenoid biosynthesis in living organisms, ca. 70% of biosynthetically active mevalonic acid (3*R*-form) is metabolized to isoprenoid lipids.

Major metabolites from [^{14}C] mevalonic acid throughout the culture cycle were phytosterols and non-polar lipids, presumably fatty acid esters of phytosterols and/or other terpenoids[6]. The ratio of radioactivity in free phytosterols and triterpenes to the total administered activity is also shown in **Fig. 1**. Phytosterol synthesis increased in parallel with cell proliferation (and radioactivity label in $CHCl_3$ layers), and then declined after the cell growth reached its stationary phase. In contrast, radioactivity in free triterpenes was negligible in young cells and only detectable in 4- and 6-week-old cells. Reverse-phase HPLC separation combined with liquid scintillation counting revealed that the radioactivity in triterpenes distributed mainly in intermediates of phytosterol biosynthesis (cycloartenol and 24-methylenecycloartanol) but rarely in tirucallol (data not shown). Thus the label in triterpenes in the later stage of the culture is not likely to be the result of metabolic changes from phytosterol pathway to other triterpenoid branches. Apparently this is in contrast to growth-dependent[7], morphogenesis-accompanied[6] or elicitor-induced[8] metabolic changes from phytosterols to other triterpenoids indicated in some plant tissue cultures.

No detectable radioactivity was found on TLC in the area corresponding to quassinoids (quassin

and neoquassin) throughout the experiment.

These results indicate, on the one hand, that undifferentiated *P. quassioides* cells which do not accumulate quassinoids do not biosynthesize the intermediate triterpenes either. On the other hand, very active phytosterol synthesis and its correlation to cell growth have been demonstrated. In order to explore the possibility of quassinoid production in cultured cells, hormonal control of the cultures and utilization of specific inhibitors of phytosterol biosynthesis are envisaged. These lines of investigation are now in progress in our laboratories.

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《和文要約》

ニガキ (*Picrasma quassioides*) 培養細胞の成長とイソプレノイド代謝

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ニガキ振盪培養の成長と [2-^{14}C] メバロン酸からのイソプレノイド生合成の関連を調べた．6週間の培養期間中，培地から細胞への放射能の取り込み，細胞のクロロホルム分画中の放射能，フィトステロールの標識は，いずれも細胞の増殖に伴って増加し，定常期に達した後は減少した．トリテルペンへの取り込みは3週間までは検出されず，培養後期に僅かに見出された．クアシノイドの標識は観察されなかった．