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Studies on the Production of Bioactive Substances

—Callus Culture of Rehmanniae Radix—

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Abstract—The rate of growth and production of bioactive substances from *Rehmannia glutinosa* Liboschitz (Scrophulariaceae) were studied with the variation on the constituents of the culture media. The best growth was observed from MS basal medium containing 3.0 ppm NAA and 2.0 ppm kinetin. Carbohydrates (fructose, glucose and sucrose), phytosterols(β-sitosterol, campesterol and stigmasterol) and carotenoid like substances were identified by GC-MS and TLC from the callus mass. However, catalpol was not detected from both solid and cell suspension cultures containing geraniol. Callus cultured *Rehmannia glutinosa* in the MS basal medium containing 0.1 ppm NAA and 0.1 ppm kinetin become differentiated to root.

Keywords—*Rehmannia glutinosa* • callus culture • cell suspension culture • fructose • glucose • β-sitosterol • campesterol • stigmasterol • carotenoids

Introduction

Rehmannia glutinosa Liboschitz is a perennial herb of the family Scrophulariaceae. This plant is native in northern part of China and is also cultivated in Korea, China and Japan for medicinal uses.

The root(*Rehmanniae Radix*) is one of the most important crude drugs used as tonic, antianemic, antipyretic and antidiabetes and it is prescribed in many herbal preparations.

Due to the different processing methods, *Rehmanniae Radix* is classified into three types (names in Korean) as Seng-Jihwang(fresh root), Koun-Jihwang(dried root) and Suk-Jihwang (steamed root), which are used in different purposes respectively in oriental medicine.[1]

It is well known that *Rehmanniae Radix* contains carbohydrates, amino acids, iridoids, phytosterols and carotenoids.[2~4,7] The chemical compositions are different with the differently processed preparations, however the relationship between those components and the pharmacological usages of the plant remains obscure.[3,4]

In the paper, we report the production of the bioactive substances from the callus cultured *Rehmannia glutinosa*.

Materials and Methods

Induction of callus: Roots and young shoots of this plant were soaked in 1~2% sodium hypochloride solution for 5~10 min, washed with sterilized double distilled water and cut into 0.5~1 cm plantlet with sterilized knife. These plantlets were cultured on the surface of MS medium containing 0.5~1.0 ppm 2.4-D to induce callus.

Growth rate of callus mass—Induced callus

of *Rehmannia glutinosa* was cultured on several MS media containing NAA and kinetin with various combination of different concentrations in dark at 25±1°C for four weeks to give callus mass. Growth rate of callus mass was calculated by weighing callus mass and that of each sample was compared.

Production of callus mass—Callus from this plant was subcultured in MS medium containing 3.0 ppm NAA and 2.0 ppm kinetin at 25±1°C in dark at four weeks intervals to produce callus mass, which was harvested.

Chemical constituents of callus mass—Fresh callus mass was extracted with 50°C hot methanol three times for three hours and filtered. Filtrate was evaporated under vacuum to give methanol extract, which was suspended in water and partitioned with ethylether and then with *n*-butanol to prepare ether extract (Fr-I), *n*-butanol extract (Fr-II) and aqueous layer (Fr-III). Fraction I was eluted with $CHCl_3$-MeOH (7 : 3) and fraction II, with $CHCl_3$-MeOH (3 : 2) on silica gel column to give compound A, B and C which were identified by GC-MS spectrum. Sugars were identified from fraction III by silica gel and cellulose thin layer chromatography and gas chromatography.

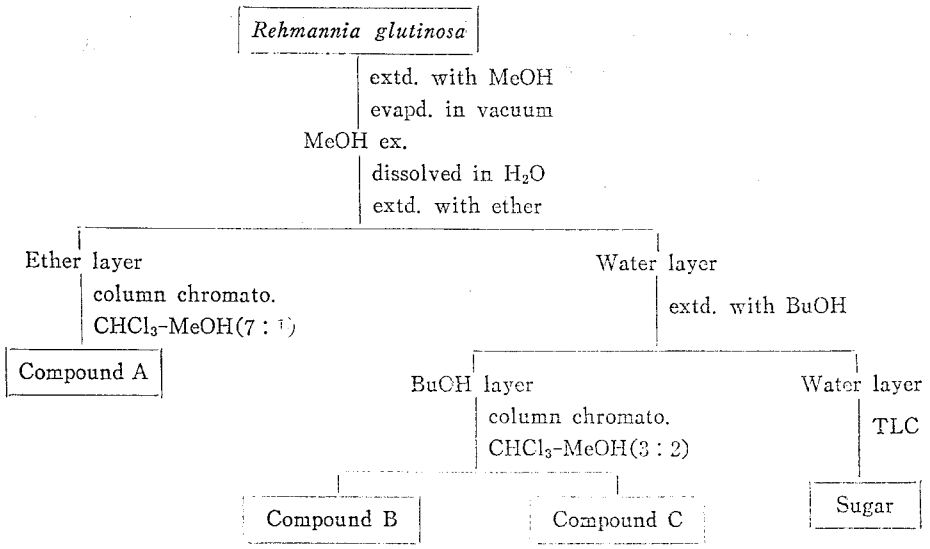
Iridoids formation by geraniol—Geraniol with various concentrations, which is one of the precursors on biosynthesis of iridoids, was added to MS medium containing 3.72 ppm NAA and 2.10 ppm kinetin. Callus was subcultured on these media at four weeks intervals to yield callus mass, which was examined on potentiality of iridoids formation.

Iridoids formation in cell suspension culture—Callus was suspended in liquid MS medium without agar and was subcultured in rotary shaker under the same conditions as mentioned above. Cell mass and filtrate obtained from each samples were examined to detect iridoids.

Differentiation of root—Callus was cultured on MS medium containing phytohormone with various concentrations in order to examine medium component leading to differentiation of root.

Results and Discussion

Callus was induced from the roots and young shoots of *Rehmannia glutinosa* Liboschitz on



Scheme I. Extraction and isolation of compounds

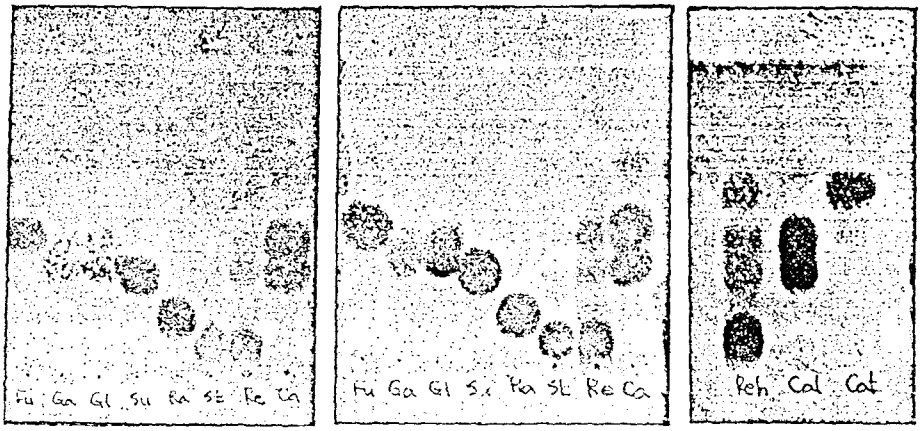


Fig. 1. TLC Chromatogram of extract of fresh *Rehmanniae Radix* and callus mass
Re: fresh *Rehmanniae Radix*
Ca: callus mass
Developer: $CHCl_3$-MeOH-H_2O (6 : 4 : 1)
Detection reagent: α-naphthol (Left), naphthoresorcinol (Right)
Detection reagent: 10% H_2SO_4
Reh: fresh *Rehmanniae Radix*
Cal: callus mass
Cat: catalpol

MS medium containing 0.5~1.0 ppm 2,4-D.

Callus subcultured on MS medium with 3.0 ppm NAA and 2.0 ppm kinetin in dark at 25± 1°C was propagated most vigorously.

Fructose, glucose and sucrose were identified and the mixture of β-sitosterol, campesterol and stigmasterol were isolated and identified from the fractions which were partitioned from methanol extract of 2.53 kg fresh callus mass of *Rehmannia glutinosa*.

Catalpol was not produced on solid and cell suspension culture of callus from *Rehmannia glutinosa* (Scheme I).

Differentiation of the root was occurred on MS medium containing 0.1 ppm NAA and 0.1 ppm kinetin.[5~7]

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