

[Phytochemistry, 58, 671-676 (2001)]

[Lab. of Pharmacognosy]

**8-Dimethylallylnaringenin 2'-Hydroxylase, the Crucial Cytochrome P450 Mono-Oxygenase for Lavandulylated Flavanone Formation in *Sophora flavescens* Cultured Cells.**

Hirobumi YAMAMOTO, Atsushi YATOU and Kenichiro INOUE\*

8-Dimethylallylnaringenin (8-DMAN) 2'-hydroxylase, which is indispensable for the formation of a lavandulylated flavanone, sophoraflavanone G, was detected in cell suspension cultures of *Sophora flavescens*. The enzyme catalyzes the 2'-hydroxylation of 8-DMAN to leachianone G, and is tightly bound to membrane. It required NADPH and molecular oxygen as cofactors, and was inhibited several cytochrome P450 inhibitors such as carbon monoxide and cytochrome c, indicating that the reaction is mediated by a cytochrome P450 monooxygenase. The optimum pH of 8-DMAN 2'-hydroxylase was 8.5, and the enzyme hydroxylated only 8-DMAN. Apparent Km values for 8-DMAN and NADPH of the enzyme were 55 and 34  $\mu$ M, respectively.

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[Lab. of Pharmacognosy]

**Glycosides of Benzyl and Salicyl Alcohols from *Alangium chinense*.**Atsuko ITOH, Takao TANAHASHI, Naotaka NAGAKURA, Kenichiro INOUE,\*  
Hiroshi KUWAJIMA and Hua-Xin WU

From the water-soluble fraction of the dried leaves of *Alangium chinense*, three new glycosides, benzyl alcohol  $\beta$ -D-glucopyranosyl-(1-2)-[ $\beta$ -D-xylopyranosyl-(1-6)]- $\beta$ -D-glucopyranoside, 2'-O- $\beta$ -D-glucopyranosylsalicin, and 2'-O- $\beta$ -D-glucopyranosyl-6'-O- $\beta$ -D-xylopyranosylsalicin were isolated along with seven known glycosides. The structures of the new compounds were determined by spectroscopic and chemical means.

[Biosci. Biotechnol. Biochem., 65, 853-860 (2001)]

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**Increases of Secondary Metabolite Production in Various Plant Cell Cultures by Co-cultivation with Cork.**Hirobumi YAMAMOTO, Atsushi YATO, Kazufumi YAZAKI, Hiroaki HAYASHI,  
Goro TAGUCHI and Kenichiro INOUE\*

Cork tissues increased secondary metabolite production of various plant cell cultures in a different manner from those of conventional elicitors. In *Sophora flavescens* and *Glycyrrhiza glabra* cultured cells, cork tissues increased the amounts of both lipophilic and hydrophilic flavonoids without affecting the cell growth, although elicitors such as copper ion and yeast extracts showed a clear inhibition of cell growth with the increasing amount of these lipophilic ones. The validity of this effect of cork tissues covered a wide range of aromatic compounds produced by suspension cultures derived from diverse plant species. Woody tissues of Japanese cypress had a very similar effect to that of cork. Partial purification of cork tissues suggested that the production-stimulating factor was present in the hemicellulose B fraction that was not included in the dedifferentiated cultured tissues.

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[Lab. of Pharmacognosy]

**Cloning and Characterization of a cDNA Encoding  $\beta$ -Amyrin Synthase Involved in Glycyrrhizin and Soyasaponin Biosyntheses in Licorice.**Hiroaki HAYASHI,\* Pengyu HUANG, Ara KIRAKOSYAN, Kenichiro INOUE, Noboru HIRAOKA,  
Yasumasa IKESHIRO, Tetsuo KUSHIRO, Masaaki SHIBUYA and Yutaka EBIZUKA

An oxidosqualene cyclase cDNA, termed GgbAS1, was isolated from cultured cells of licorice (*Glycyrrhiza glabra*) by heterologous hybridization with cDNA of *Arabidopsis thaliana* LUP1 lupeol synthase. The yeast transformed with an expression vector containing the open reading frame of GgbAS1 produced  $\beta$ -amyrin, indicating that GgbAS1 encodes  $\beta$ -amyrin synthase involved in the glycyrrhizin and soyasaponin biosyntheses in licorice. Northern blot analysis showed that the level of  $\beta$ -amyrin synthase mRNA was drastically changed in the cultured licorice cells, whereas the mRNA level of cycloartenol synthase was relatively constant