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Biotransformation of Glycyrrhizin by Buffered Cell Suspension Cultures of *Catharanthus roseus*

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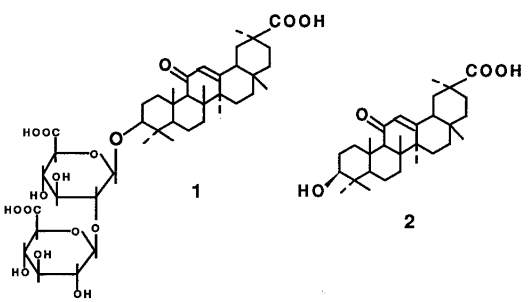
Since glycyrrhizin is an anti-allergy agent and acts as an adrenocortical hormone in the human, its biotransformation by bacteria and plant cells is of considerable interest. Hattori *et al.*[1] have studied the metabolism of glycyrrhizin by human intestinal bacteria. However, there are no reports on the biotransformation of glycyrrhizin by plant tissue cultures.

We have studied the biotransformation of glycyrrhizin by buffered cell suspension cultures of *Catharanthus roseus* and we outline here some of our preliminary results in which the buffered cell suspension cultures of *C. roseus* are able to convert glycyrrhizin (1) to glycyrrhetic acid (2) with a 38 % yield.



Materials and Methods

Analytical and prep. TLC were performed on Merk GF_{254} silica gel plates with 0.50 and 1.00 mm layers of absorbance, respectively. Detection of components was first by UV (254 nm) absorbance, followed by spraying with 10 % H_2SO_4 and heating the plates at 100° for 5 min.

Substrate. Glycyrrhizin (1) (98 %, Nacalai tesque) was used without further purification. Glycyrrhetic acid (2) (99 %, Aldrich) was obtained commercially.

Fast atom bombardment mass spectrometry. FAB-MS of the biotransformation products was carried out on a high-resolution mass spectrometer (JEOL DX 303HF) at an accelerating voltage of 3 kV and a resolution of 1 : 2000. For calibration, perfluoroalkyl phosphazine cluster ions were used. A portion of the products was dissolved in glycerol and the solution applied to a stainless steel probe tip. The JEOL FAB gun was operated at 6 kV with xenon as the FAB gas.

Incubation of the substrate with buffered cell suspension cultures of *C. roseus*. Buffered cell suspension cultures of *C. roseus* were prepared in 300 m*l* conical flasks, each containing 100 m*l* of

buffered solution. The feeding experiment was described in our previous paper[3].

Isolation and identification of product. The cultured mixture was worked-up in a similar manner to that described by Hamada, *et al.*[2,3] The yield of the product was determined on the basis of the peak area on HPLC and expressed as a relative percentage of the total amount of the whole reaction mixture extracted.

Results and Discussion

Periwinkle callus tissues were derived from the leaves of *Catharanthus roseus* in June of 1990. The feeding experiment and the work-up were carried out as described in our previous paper[2,3].

Glycyrrhizin (1) was converted to glycyrrhetic acid (2) with a 38 % yield after 2 days incubation. Characterization of the product (2) was carried out by EI and FAB-MS [M^++1 471]. The molecular weight of 2 was 470. The absolute configuration of the predominant isomers of 2 was β. The identification of 2 was confirmed by a comparison of TLC, HPLC, IR and FAB-MS data with that obtained using an authentic sample of glycyrrhetic acid. The formation of 2 indicates the buffered cell suspension cultures of *C. roseus* hydrolyzed glycyrrhizin to glycyrrhetic acid. Such a glycoside hydrolysis has not been observed in the biotransformation of glycoside such as digitoxin and β-methyl-digitoxin with plant cell suspension cultures[4].

Thus, it was found that the buffered cell suspension cultures of *C. roseus* have the ability to transform glycyrrhizin to glycyrrhetic acid.

Work is in progress to isolate the enzyme of a glycoside hydrolysis from *C. roseus* cell suspension cultures.

Acknowledgement

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References

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《和文要約》

緩衝溶液で培養したニチニチソウ培養細胞によるグリチルリチンの生化学的物質変換

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緩衝溶液で培養したニチニチソウ培養細胞は，2日間の培養時間で，グリチルリチンをグリチルレチン酸に38％の収量で変換することがわかった．