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# GLUCOSYLATION OF SALICYL ALCOHOL IN CULTURED PLANT CELLS

HAJIME MIZUKAMI, TOSHIMITSU TERAO, HIROSHI MIURA and HIROMU OHASHI

Faculty of Pharmaceutical Sciences, Nagasaki University, Nagasaki-shi, Japan

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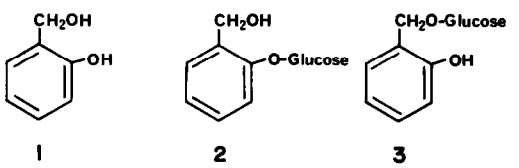
**Key Word Index**—Biotransformation; glucosylation; plant suspension cultures; salicyl alcohol; salicin; isosalicin.

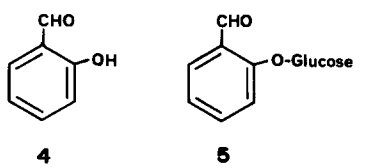
**Abstract**—Salicin and isosalicin were identified as a product of glucosylation of salicyl alcohol in *Lithospermum erythrorhizon* suspension cultures while in *Datura innoxia* suspension cultures only isosalicin was isolated as a glucosylation product. In suspension cultures from seven different plant species examined, *Gardenia jasminoides* and *L. erythrorhizon* cultured cells could produce salicin from salicyl alcohol while others produced predominantly isosalicin.

## INTRODUCTION

Biosynthesis of salicin (**2**) has been of interest because exogeneously supplied salicyl alcohol (**1**) was shown to be converted not to salicin but to *o*-hydroxybenzyl-β-D-glucoside (isosalicin, **3**) in various plant materials both *in vivo* and *in vitro* [1]. Zenk [2] reported that salicin was biosynthesized from salicylaldehyde (**4**) via helicin (**5**) and suggested that salicyl alcohol, therefore, is not a direct precursor of salicin. Tabata *et al.* [3] also showed that in *Datura innoxia* suspension cultures salicyl alcohol was glucosylated to form isosalicin.

In this report we describe the formation of salicin from salicyl alcohol in suspension cultures of *Lithospermum erythrorhizon* and *Gardenia jasminoides*, suggesting that salicyl alcohol may be a direct precursor of salicin in these cultured cells.




## RESULTS AND DISCUSSION

### *Glucosylation product of salicyl alcohol in* Datura innoxia *suspension cultures*

One glucosylation product (SG-1) was isolated from *D. innoxia* cultured cells supplied with salicyl alcohol ($5 \times 10^{-3}$ M). On enzymic hydrolysis with almond β-glucosidase, SG-1 gave salicyl alcohol and glucose in 1 : 1 molar ratio but its chromatographic properties were different from those of salicin. The UV spectrum of SG-1 showed a bathochromic shift with alkali, which indicates the presence of a free phenolic hydroxyl group. From these results, SG-1 was identified to be isosalicin [4]. This is consistent with the result obtained by Tabata *et al.* [3] who showed the formation of isosalicin from salicyl alcohol in *D. innoxia* cultured cells based on TLC and electrophoresis data. Salicin was not detected in either the culture medium or the cultured cells.

### *Glucosylation products in* Lithospermum erythrorhizon *suspension cells*

Two glucosylation products (SG-2 and SG-3) were detected on TLC with solvent system 1 ($R_f$ 0.25 and 0.33, respectively) in *L. erythrorhizon* suspension cultures supplied with salicyl alcohol. SG-2 (mp 204–205°), having the same chromatographic properties as authentic salicin on TLC and HPLC, was isolated from *L. erythrorhizon* cultured cells by prep. TLC. On enzymic hydrolysis with almond β-glucosidase, SG-2 also yielded salicyl alcohol and glucose in 1 : 1 molar ratio, but no bathochromic shift in the UV spectrum was observed. By direct comparison (UV, IR, mass spectra and $^1$H NMR) with the authentic sample, this compound was identified as salicin. SG-3 showed the same chromatographic properties on TLC and HPLC as SG-1 and is presumed to be isosalicin.

### *Glucosylation of salicyl alcohol in various cultured plant cells*

Various kinds of suspension cultures, specified in Table 1, were supplied with $5 \times 10^{-3}$ M salicyl alcohol 6 days after transfer to new medium and cultured for an additional 4 days. Glucosylation products in the cells were analysed by TLC (solvent systems 1–3) and HPLC. As shown in Table 1, among eight different suspension cultures tested, only *Gardenia jasminoides* and *L. erythrorhizon* cultured cells produced both salicin and isosalicin, while in the cultured cells of other plants isosalicin was the predominant product of glucosylation.

Quantitative analysis by HPLC showed that when 150 μmol salicyl alcohol was supplied to *G. jasminoides* suspension cultures 6 days after cell inoculation,

Table 1. Glucosylation product of salicyl alcohol in various plant cultured cells

| Plant name | Origin | Glucosylation product (%) | |
|---|---|---|---|
| | | Salicin | Isosalicin |
| *Nicotiana tabacum* | Root | 3 | 97 |
| *Datura innoxia* | Root | 0 | 100 |
| | Anther | 0 | 100 |
| *Duboisia myoporoides* | Leaf | 0 | 100 |
| *Catharanthus roseus* | Leaf | 3 | 97 |
| *Bupleurum falcatum* | Seedling | 0 | 100 |
| *Gardenia jasminoides* | Leaf | 69 | 31 |
| *Lithospermum erythrorhizon* | Seedling | 81 | 19 |

27.6 μmol salicin and 14.1 μmol isosalicin were formed in the cells within 4 days, and 2.2 μmol salicin and 0.3 μmol isosalicin were found in the medium, which means *ca* 30% of supplied salicyl alcohol was converted to the glucosides.

The present study shows that in the cultured cells of some plants salicin can be formed from exogeneously supplied salicyl alcohol. It is of interest to note that the existence of cultured cells having the capability to produce salicin from salicyl alcohol may aid in the elucidation of the detailed biosynthetic pathway of salicin in cell-free systems.

## EXPERIMENTAL

*Suspension cultures.* Plant suspension cultures were maintained in Linsmaier–Skoog's basal medium [5] supplemented with $10^{-5}$ M IAA and $10^{-6}$ M kinetin (for *Nicotiana tabacum*), with $10^{-6}$ M 2,4-D (for *Datura innoxia*) or with $10^{-6}$ M 2,4-D and $10^{-6}$ M kinetin (for others) at 25° under dim light. Salicyl alcohol ($5 \times 10^{-3}$ M) was administered aseptically through a membrane filter (0.45 μm) to suspension cultures in 100-ml conical flasks containing 30 ml medium 6 days after cell inoculation and the cells were cultured for an additional 4 days.

*Extraction and qualitative analysis of glucoside.* The cells were homogenized with MeOH and refluxed twice for 1 hr each. Glucoside fractions were obtained by extracting the MeOH extract, or the medium, with *n*-BuOH satd with $H_2O$ and were analysed by Si gel TLC with the following solvent systems: (1) EtOAc–MeOH (90:10); (2) EtOAc–MeOH–$H_2O$ (65:22:13); (3) EtOAc–*iso*-PrOH–$H_2O$ (65:24:11), and by HPLC (Waters) with a Radial-Pak A (Waters) column eluted with MeOH–$H_2O$ (25:75) at a flow rate of 1.5 ml/min.

*Isolation of glucoside.* The glucoside fractions from *D. innoxia* and *L. erythrorhizon* cells supplied with salicyl alcohol were subjected to prep. TLC on Si gel $GF_{254}$ with solvent system 1 for development and were eluted with MeOH. This afforded SG-1 (24 mg) from *D. innoxia* cells and SG-2 (17 mg) and SG-3 (5 mg) from *L. erythrorhizon* cells.

*Enzymic hydrolysis of glucoside.* The hydrolysis of glucosides was carried out with almond β-glucosidase (Sigma). The products were analysed by TLC and HPLC for detection of salicyl alcohol and by PC [6] and an enzymic method [7] for glucose.

*Quantitative assay of salicin, isosalicin and salicyl alcohol.* The amounts of salicyl alcohol and its glucosides were estimated by HPLC (Tri-Rotar II, Japan Spectroscopic Co.) at 270 nm with a Finepak SIL $C_{18}$ (Japan Spectroscopic Co.) column with a solvent system of MeOH–$H_2O$ (25:80) at a flow rate of 1.5 ml/min. Retention times: salicin, 4.9 min; isosalicin, 6.8 min; salicyl alcohol, 8.6 min.

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