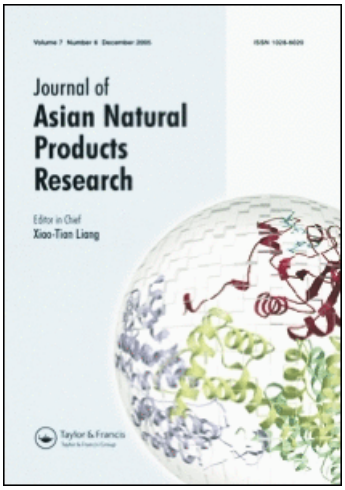

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GLUCOSYLATION OF SALICYL ALCOHOL BY CELL SUSPENSION CULTURES OF *SOLANUM LACINIATUM*

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Cell suspension cultures of *Solanum laciniatum* were able to transform exogenously inoculated salicyl alcohol into salicyl alcohol 7-O-β-D-glucopyranoside (isosalicin). The highest level of isosalicin (54.6 mg/g dry weight) in the cells was formed within 2 days after inoculation with salicyl alcohol (37.5 mg/flask containing 50 ml of medium). The biotransformation capacity of the cell suspension cultures was about 31.1%.

Keywords: *Solanum laciniatum*; Biotransformation; Bioconversion; Glucosylation; Salicyl alcohol; Isosalicin

INTRODUCTION

Plant cells cultures can be used not only for the production of secondary metabolites but also for the biotransformation of various compounds. Various plant cell cultures are capable of glycosylating a variety of exogenously supplied compounds [1]. The glucosylation of simple phenols (e.g. salicylic acid and salicyl alcohol) by some cell suspension cultures have been reported by Umetami *et al.* [2]. Mizukami *et al.* [3] have reported the

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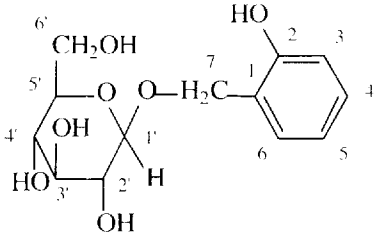
formation of salicin from salicyl alcohol in cell suspension cultures of *Lithospermum erythrorhizon* and *Gardenia jasminides*, and Dombrowksi [4] has reported the transformation of salicyl alcohol into salicin and isosalicin in cell suspension cultures of *Salix matsudana*.

Although cell suspensions of *solanum* species (e.g. *Solanum aviculare*, *Solanum mammosum*) did not produce solasodine [5,6], the cultures can transform some exogenously inoculated substrates. Cell suspension cultures of *Solanum aviculare* have been shown to be able to transform some exogenously inoculated natural and synthetic substances, e.g. (+)-3-carene, citronella, thujol, thujone, *cis* and *trans* verbenol, verbenone and (−)-limonene [7]. Callus cultures of *Solanum mammosum* can transform progesterone into 5-pregnene-3,20-dione [8]. Syahrani *et al.* [9,10] have reported that cell suspension cultures of *Solanum mammosum* have the ability to transform salicylamide into salicylamide 2-O-β-D-glucopyranoside and salicyl alcohol into salicyl alcohol 2-O-β-D-glucopyranoside (salicin).

In this report, we describe the formation of isosalicin (**1**) from exogenously supplied salicyl alcohol (**2**) in the cell suspension cultures of *Solanum laciniatum* (see Fig. 1).



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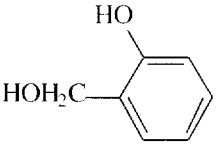

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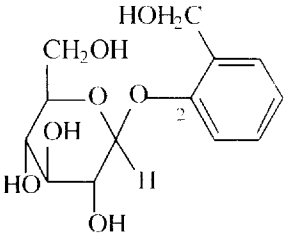

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FIGURE 1 The structure of isosalicin (**1**), salicyl alcohol (**2**), and salicin (**3**).

RESULTS AND DISCUSSION

The toxicity of **2** towards *Solanum laciniatum* cell suspension cultures was investigated for concentrations in the range 500–1500 mg/l. Cell death was observed at higher concentrations (1500, 1250 and 1000 mg/l). At concentrations lower than 750 mg/l, cells survived but did not grow. In all of the experiments, 750 mg/l of **2** was administered. The foregoing experiments indicated that cell suspension cultures of *Solanum laciniatum* were able to tolerate a lower concentration of **2**, as substrate, than was the case for cell suspension cultures of *Solanum mammosum* [9,10].

TLC analysis showed the presence of metabolite **1** (R_f 0.32) in cell extracts. Two control experiments showed that in the absence of cells, **2** (R_f 0.70) remained unchanged in the control medium, and **1** was only produced when **2** was added to the suspension cultures. The metabolite **1** exhibited a similar R_f value and UV absorbance reflectance spectrum to that determined for an authentic specimen of salicin (**3**) (Sigma).

The ^{13}C NMR spectrum of **1** was similar to that of **3** [10], except for the occurrence of the aryl methylene signal at 65.0 ppm. This signal exhibited a downfield shift of about 7 ppm, compared with that of the equivalent resonance of **3** [10]. Five of the methine and one of the methylene signals could be assigned to a glucopyranosyl unit (see Table I). The ^1H NMR spectrum of **1** included a signal at 4.33 ppm (d, $J = 7.7$ Hz) attributable to a β-oriented anomeric methine proton. HMBC data (Table I) established that the β-glucopyranosyl unit was attached to C-7. Irradiation of the glucosyl H-1′ resonance in a NOE-difference experiment enhanced H-6 (7.45 ppm), H-7A (4.64 ppm), H-3′ (3.22 ppm) and H-5′ (3.19 ppm), thereby confirming **1** to be a 7-O-β-glucopyranoside (isosalicin). A complete assignment of the ^1H and ^{13}C NMR spectra of **1**, determined in DMSO-d_6 is presented in Table I. To our knowledge, this is the first complete assignment of the ^1H and ^{13}C NMR resonances of isosalicin.

The time course of formation of **1** from **2** in cell suspension cultures of *Solanum laciniatum* is shown in Fig. 2. Quantitative analyses showed that the greatest level of **1** (28.9 mg/flask equivalent to 54.6 mg/g dry weight) was formed in the cells 2 days after the inoculation of 37.5 mg of **2** in a flask containing 50 ml of medium each (i.e. an inoculation level 750 mg/l). Glucoside **1** was not detected in the medium during the 14 day incubation period. On the other hand, salicyl alcohol was detected in the medium during the first 2 days of the incubation period.

The maximum glucosylation capability of cell suspension cultures of *Solanum laciniatum* reported here, namely a 31.1% conversion **2** to **1**, is

TABLE I ^{1}H and ^{13}C NMR spectral data determined for compound **1**

C	^{13}C	$^{1}H^{a}$	*H–H COSY*	*NOE*	^{1}H ^{13}C *HMBC*
C-1	124.4				
C-2	154.5				
		9.45 (br s, OH)			
C-3	114.8	6.86 (m)	H-4, H-5		C-1, C-2, C-5
C-4	128.1	7.16 (ddd, 1.8, 7.4, 8.0)	H-3, H-5, H-6	H-3, H-5	C-2, C-6
C-5	118.7	6.85 (m)	H-4, H-6, H-3		C-2, C-3
C-6	128.6	7.45 (dd, 1.7, 7.5)	H-5, H-4	H-5, H-7A, H-7B, H-1′	C-2, C-4, C-7
C-7	65.0	4.64 (d, 12.9)	H-7B	H-6, H-7B, H-1′	C-1′, C-1, C-2, C-6
		4.86 (d, 12.9)	H-7A	H-6, H-7A	C-1′, C-1, C-2, C-6
C-1′	102.4	4.33 (d, 7.7)	H-2′	H-6, H-7A, H-3′, H-5′	C-2′, C-7, C-3′ C-5′
C-2′	73.6	3.12 (m, Hax)	H-1′, H-3′, 2′-OH		
		5.12 (br d, 4.6 OH)	H-2′		
C-3′	76.8	3.22 (m, Hax)	H-2′, H-4′, 3′-OH		
		4.98 (br d, 4.6 OH)	H-3′		
C-4′	70.2	3.18 (m, Hax)	H-3′, 4′-OH		
		4.95 (br d, 4.8 OH)	H-4′		C-3′ C-5′, C-4′
C-5′	76.9	3.19 (m)	H-6′A		
C-6′	61.0	3.55 (ddd, 5.7, 5.7, 11.7)	H-5′, H-6′B, 6′-OH		C-4′, C-5′
		3.78 (ddd, 1.9, 5.7, 11.7)	H-6′A, 6′-OH		C-4′
		4.58 (br t, 5.8 OH)	H-6′A, H-6′B		C-5′, C-6′

[a] Signal multiplicities and coupling constants (Hz) are given in brackets.

essentially the same as that reported previously for *Gardenia jasminoides* (30%) [3], but significantly lower than that found for *Salix matsudana* (48%) [4] and *Solanum mammosum* (51%) [10]. To our knowledge this is the first report of the biotransformation of salicyl alcohol into its glucoside **1** (isosalicin) by cell suspension cultures of *Solanum laciniatum*. Our results showed that cell suspension cultures of *Solanum laciniatum* can glucosylate exogenously introduced substrates. Some biotransformation studies using other exogenous substrates are in progress in our laboratory.

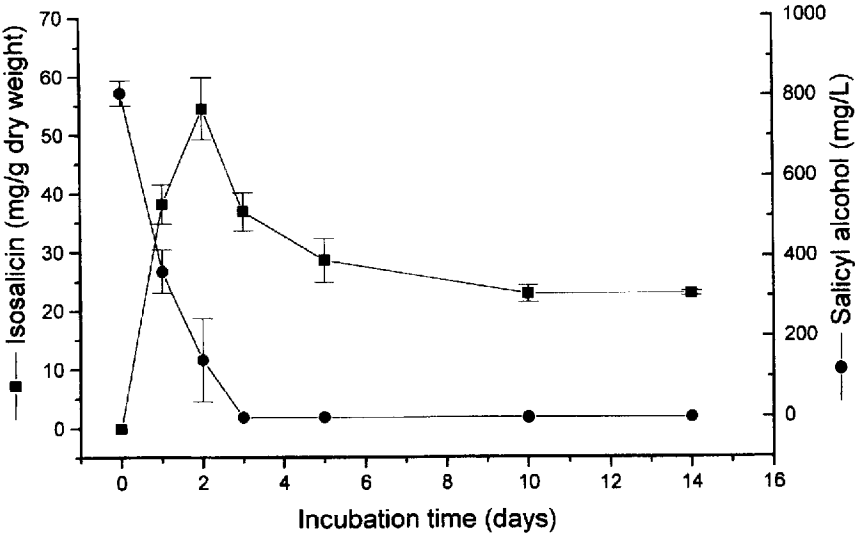

FIGURE 2 The time course of the biotransformation of salicyl alcohol (**2**) to isosalicin (**1**) by cell suspension cultures of *Solanum laciniatum*. Values represent mean ± SD ($n = 4$).

EXPERIMENTAL SECTION

General Procedures

The UV absorbance reflectance spectrum were recorded on a Shimadzu CS-930 TLC-Scanner. ^{1}H (400.13 MHz) and ^{13}C NMR (100.26 MHz), and COSY, ROESY, HMBC and HMQC spectra of **1** were determined in DMSO-d_6 using a Bruker DRX 400 spectrometer. HMBC and HMQC (phase sensitive) spectra were obtained with gradient selection. NOE-difference spectra were determined at 300.13 MHz using a Bruker AC 300 spectrometer.

Cell Suspension Cultures

Cell suspension cultures were initiated from previously established shoot cultures of *Solanum laciniatum* Ait. (code sl-4) provided by Prof. Dr. A.W. Alfermann, University of Düsseldorf, Germany. The suspension cultures were cultivated in 300 ml Erlenmeyer flask containing 50 ml of modified Murashige and Skoog medium [11] supplemented with sucrose (30 g/l),

kinetin (2 mg/l), NAA (1 mg/l) and casein hydrolisate (1 g/l). Flasks were shaken on a rotary shaker (120 rpm) at 25 + 1°C under continuous light (ca. 2000 lx).

Toxicity and Biotransformation Experiments

Cells (10 g fresh weight) were inoculated into liquid medium (50 ml) without **2** (control culture) and into liquid medium containing various concentration of **2** (500, 750, 1000, 1250 and 1500 mg/l). After 7 days incubation the cultures were harvested, collected, filtered, weighed, dried and powdered. The standard protocol for the biotransformation experiment was as follows: cells (10 g fresh weight) were inoculated into a 300 ml Erlenmeyer flask containing 50 ml of medium, **2** (750 mg/l) was added and the mixture cultured for 1–7 days.

Extraction, Isolation and Purification of 1

The oven dried (40°C) powdered biomass (9.75 g) collected from 15 Erlenmeyer flasks was refluxed (2 h) in MeOH. The MeOH extract was concentrated under reduced pressure to afford a residue (2.13 g) which was submitted to silica gel 40 (70–230 Mesh ASTM) (E. Merck) column chromatography and preparative TLC using silica gel 60 GF 254 (E. Merck) (Solvent system: EtOAc/MeOH/H_2O 77 : 13 : 10) to give **1** (36 mg).

Quantitative Analysis of 1 and 2

The concentration of **1** and **2** in the medium and the biomass were determined densitometrically using a Shimadzu CS 930 TLC scanner and silica gel 60 GF 254 precoated plates (E. Merck) eluted with EtOAc/MeOH/H_2O 77 : 13 : 10. Quantitation was performed by measuring the absorbance reflectance (at 275 nm for **1**; 278 nm for **2**) of the analyte spots. The concentrations of **1** and **2** were determined from calibration graphs using an authentic specimen of salicyl alcohol (Sigma) and isolated isosalicin, as external standards on the same plate. With this method, linearity of response were achieved for loadings of 0.5–8.0 µg/spot (for isosalicin) and 0.8–20 µg/spot (for salicyl alcohol) respectively. The results of recovery studies were 108.3% (for isosalicin); 104.2% (for salicyl alcohol), with a standard deviation of 2.4% (isosalicin; $n = 4$) and 2.15% (salicyl alcohol; $n = 4$).

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References

[1] Suga, T. and Hirata, T., *Phytochemistry*, 1990, **29**, 2393–2406.
[2] Umetami, Y., Tanaka, S. and Tabata, M., in: "*Plant Tissue Organ Culture 1982*," Fujwara, A., ed., Proceedings of the 5th International Congress Plant Tissue Cell Culture, Marzen Company, Tokyo, 1982, pp. 382–384.
[3] Mizukami, H., Terao, T., Miura, H. and Ohashi, H., *Phytochemistry*, 1983, **22**, 679–680.
[4] Dombrowski, K., Phytochemische und enzymologische Untersuchungen zur Biotransformation von Salicylverbindungen durch Zellkulturen der Weidenart *Salix matsudana f. tortousa*, Dissertation, Universität Düsseldorf, Düsseldorf, Germany, 1993.
[5] Galenes, I.T., Webb, D.T. and Rosario, O., *J. Nat. Prods.*, 1984, **47**, 373–376.
[6] Indrayanto, G., Isnaeni and Sutarjadi, *Plant. Med.*, 1986, **42**, 413.
[7] Vanek, T., Valterova, I., Macek, T., Budesinsky, M. and Vasickova, S., *Plant. Med.*, 1990, **56**, 628–629.
[8] Sondakh, R., Biotransformasi progesteron oleh kultur kalus *Solanum mammosum*, MSc Thesis, Faculty of Graduate Student, Airlangga University, Surabaya, Indonesia, 1989.
[9] Syahrani, A., Indrayanto, G., Sutarjadi and Wilkins, A., *Chem. Pharm. Bull.*, 1997, **43**, 555–557.
[10] Syahrani, A., Indrayanto, G., Wilkins, A. and Sutarjadi, *Nat. Prod. Sci.*, 1997, **3**, 71–74.
[11] Murashige, T. and Skoog, F., *Physiol. Plant*, 1962, **15**, 473–497.