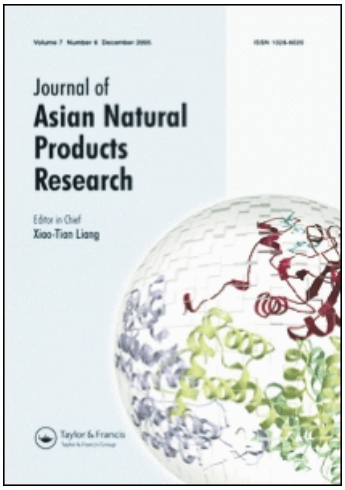

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

High yield formation of o -aminobenzoic acid-7- O -β- d -(β-1,6- O - d -glucopyranosyl)-glucopyranosyl ester in cell suspension cultures of Solanum mammosum

Lanny Hartanti[a]; Ivy Widjaja[a]; Achmad Syahrani[a]; Gunawan Indrayanto[a]
[a] Laboratory of Pharmaceutical Biotechnology, Faculty of Pharmacy, Airlangga University, Surabaya, Indonesia

Online publication date: 09 September 2010

To cite this Article Hartanti, Lanny , Widjaja, Ivy , Syahrani, Achmad and Indrayanto, Gunawan(2002) 'High yield formation of o -aminobenzoic acid-7- O -β- d -(β-1,6- O - d -glucopyranosyl)-glucopyranosyl ester in cell suspension cultures of Solanum mammosum', Journal of Asian Natural Products Research, 4: 1, 61 — 65
To link to this Article: DOI: 10.1080/10286020290019712
URL: http://dx.doi.org/10.1080/10286020290019712

Journal of Asian Natural Products Research, 2002 Vol. 4 (1), pp. 63–67

HIGH YIELD FORMATION OF *O*-AMINOBENZOIC ACID-7-*O*-β-D-(β-1,6-*O*-D-GLUCOPYRANOSYL)-GLUCOPYRANOSYL ESTER IN CELL SUSPENSION CULTURES OF *SOLANUM MAMMOSUM*

LANNY HARTANTI, IVY WIDJAJA, ACHMAD SYAHRANI and GUNAWAN INDRAYANTO*

Laboratory of Pharmaceutical Biotechnology, Faculty of Pharmacy, Airlangga University, Jl. Dharmawangsa Dalam, Surabaya 60286, Indonesia

(Received 26 February 2001; Revised 30 April 2001; In final form 6 June 2001)

Cell suspension cultures of *Solanum mammosum* cultivated in modified Murashige & Skoog media could synthesize *o*-aminobenzoic acid-7-*O*-β-D-(β-1,6-*O*-D-glucopyranosyl)-glucopyranosyl ester from *o*-amino benzoic acid with a yield of about 20% dry weight in 7 days. The maximum production of *o*-aminobenzoic acid-7-*O*-β-D-(β-1,6-*O*-D-glucopyranosyl)-glucopyranosyl ester was 31.8% on dry weight basis.

Keywords: Biotransformation; *o*-Aminobenzoic acid; *Solanum mammosum*; Cell suspension cultures; *o*-Aminobenzoic acid-7-*O*-β-D-(β-1,6-*O*-D-glucopyranosyl)-glucopyranosyl ester

INTRODUCTION

Various plant cell cultures are capable of glucosylating a variety of exogenous supplied substrates [1]. Cell suspension cultures of *Salix matsudana* transformed salicyl alcohol into salicin and isolacin, whilst salicylic acid was bioconverted into salicylic acid-2-*O*-β-D-glucopyranoside and salicylic acid-7-*O*-β-D-glucopyranoside [2]. High yield formation of arbutin from hydroquinone by cell suspension cultures of *Catharathus roseus* [3] and *Rauwolfia serpentina* [4] were reported. We have previously reported the bioconversion of salicyl alcohol into salicin [5], salicylamide into salicylamide 2-*O*-β-D-glucopyranoside [6].

ortho-Aminobenzoic acid (**1**) was transformed into a minor glucoside, *o*-aminobenzoic acid-7-*O*-β-D-glucopyranosyl ester (**2**) and a major glucoside, *o*-aminobenzoic acid-7-*O*-β-D-(β-1,6-*O*-D-glucopyranosyl)-glucopyranosyl ester (**3**) by cell suspension cultures of *Solanum mammosum* [7] (Fig. 1). Aminobenzoic acid derivatives, that mostly slightly soluble in water, can be used as UV absorbers in sunscreen preparations [8]. By glucosylation, the product(s) will be more water soluble and active, so their formulation into pharmaceutical preparations will be easier.

*Corresponding author. Fax: +62-31-5020514. E-mail: indrayanto@hotmail.com

ISSN 1028-6020 © 2002 Taylor & Francis Ltd
DOI: 10.1080/10286020290019712

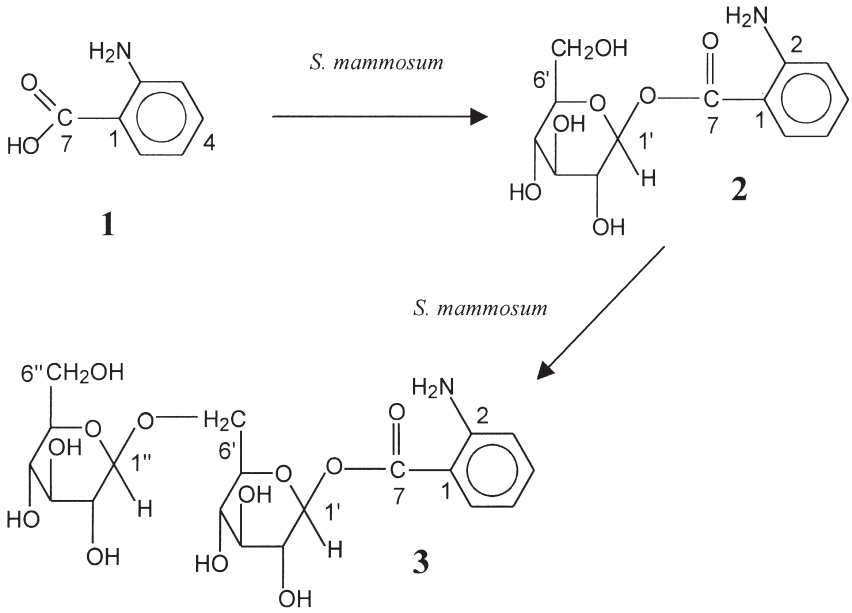

FIGURE 1 Biotransformation **1** to **2** and **3** by cell suspension cultures of *Solanum mammosum*.

In this work, we describe a high yield formation of **3** in cell suspension cultures of *Solanum mammosum* by optimizing some external factors.

EXPERIMENTAL

Cell Suspension Cultures and Biotransformation Conditions

Cell suspension cultures of *Solanum mammosum* (cod*e* LOG and IV) were cultivated in Erlenmeyer flask (300 ml) containing 50 ml of modified MS [11] medium, supplemented with sucrose (3%, w/v), kinetin (2 mg/l), 1-napthylacetic acid (1 mg/l) and casein hydrolysate (1 g/l) (control MS medium) on a gyrotary shaker (100 rpm) at 25 ± 1° under continuous light (*ca.* 1500 lux; Philips TL 54/40 W) as previously reported by Syahrani *et al.* [7]. PCV was determined according to the method of Seitz *et al.* [12].

Biotransformation experiments were typically performed by inoculating cells (10 g fresh weight) into the medium (50 ml) containing **1** (750 mg/l; Sigma, St Louis, USA) and incubated for 7 days.

In order to increase the formation of the major glucoside **3**, some external factors relating to the product formation were optimized. In this experiment, various concentrations of carbon sources, growth hormone combinations (kinetin/napthylacetic acid, kinetin/2,4 dichlorophenoxy acetic acid and kinetin/indolacetic acid), $CuSO_4 \cdot 5H_2O$, $CoCl_2 \cdot 6H_2O$, KH_2PO_4, $CaCl_2 \cdot 2H_2O$ were used instead of their original concentrations in the control MS medium. Each treatment was performed in four Erlenmeyer flasks. After 7 days, the cultures were harvested, filtered, weighed, oven dried at 40°C (until their water content was *ca.* 2%, w/w), collected, then powdered and analyzed.

To inoculate more substrate, in order to produce more glucoside, feeding experiments were performed as follows: on second or fifth day, after the cells inoculated into the medium containing sucrose (0.25%), $CuSO_4 \cdot 5H_2O$ (0.25 mg/l) and **1** (750 mg/l), more **1** (25 mg/Erlenmeyer/day) was added into the suspension cultures.

Biotransformation capacity was calculated as percentage of the total conversion of the inoculated substrate to product(s) in each Erlenmeyer flask.

Analysis of 1–3

The content of **1**–**3** in the medium and the biomass were determined densitometrically using a Shimadzu CS 930 TLC scanner and pre-coated silicagel F_{254} plates (Cat. 1.05554, E. Merck, Darmstadt, Germany) eluted with EtOAC/MeOH/H_2O 70:20:10 according to Indrayanto *et al.* [13]. Quantitation was performed by measuring the absorbance reflectance of the analyte spots at 332 nm. R_f values of **1**–**3** were 0.71, 0.53 and 0.31, respectively.

RESULTS AND DISCUSSION

Table I shows that, the formation of the glucoside was stimulated by reducing the concentration of sucrose in media compared to cultures cultivated in the control medium (3% sucrose). Our previous study reported that the maximum formation of **3** was achieved in the stationary phase [9]. Glucosylation of esculetin by cultured cells of *Gardenia jasminoides* is highest at the late stationary phase of the cell growth cycle [1]. Indrayanto *et al.* [10] also reported that by reducing the concentration of sucrose in media, the content of solasodine in shoot cultures of *Solanum laciniatum* was increased significantly. It seems that the production of the secondary metabolites in certain cell cultures (e.g. *Solanum* spp.) is achieved mostly in the stationary phase, in which the content of the sucrose in the media is relatively low. Replacing sucrose with glucose and lactose in media seems to reduce the formation of the product. Changing the combination of

TABLE I Effects of carbon sources and Cu $SO_4 \cdot 5H_2O$ on the formation of glucoside **3** in suspension cultures of *Solanum mammosum* after 7 days of incubation

Treatment		**3** (mg/g DW)*	*Biotransformation capacity*†	*% PCV*†
Sucrose‡ (%, w/v)	(0)	213.9 (±1.7)	75.1 ± 2.3	38.2 ± 1.8
	(0.25)	131.8 (±0.2)	69.9 ± 0.9	62.4 ± 3.2
	(0.50)	106.6 (±0.9)	81.3 ± 2.2	53.1 ± 1.3
	(1.0)	125.6 (±2.8)	67.1 ± 1.3	60.1 ± 4.7
	(2.0)	87.1 (±0.3)	67.6 ± 2.9	80.3 ± 4.5
	(3.0)¶	77.9 (±1.1)	59.5 ± 1.5	74.5 ± 6.3
	(5.0)	72.1 (±1.2)	65.2 ± 4.4	66.9 ± 10.0
	(6.0)	74.1 (±6.2)	41.6 ± 1.6	50.0 ± 12.8
	(8.0)	51.1 (±4.1)	29.3 ± 4.9	47.7 ± 2.7
Glucose‡ (%, w/v)	(0.5)	88.6 (±0.8)	69.8 ± 5.3	56.0 ± 2.9
	(1.0)	58.4 (±12.0)	60.6 ± 2.5	44.7 ± 2.7
	(3.0)	47.1 (±4.3)	61.7 ± 1.5	46.1 ± 3.5
	(5.0)	31.7 (±1.6)	41.6 ± 1.4	48.9 ± 3.1
	(7.0)	18.9 (±2.1)	26.5 ± 1.5	48.3 ± 2.8
Lactose‡ (%, w/v)	(3)	36.1 (±4.2)	39.0 ± 2.7	38.6 ± 3.8
$CuSO_4 \cdot 5H_2O$§ (mg/l)	(0.025)¶	41.7 (±3.0)	34.5 ± 2.8	52.9 ± 3.4
	(0.125)	56.8 (±8.1)	41.1 ± 2.3	54.7 ± 3.8
	(0.25)	61.1 (±2.1)	46.4 ± 2.7	48.3 ± 4.7
	(0.5)	55.4 (±2.0)	43.6 ± 1.3	49.3 ± 2.4
	(2.5)	59.2 (±3.0)	43.8 ± 2.8	47.4 ± 2.4

* Results are expressed as mean of duplicate analysis; % deviation from mean are given in parentheses.
† Values expressed as mean ± SD ($n = 4$).
‡ Using suspension cultures code LOG.
¶ Control medium.
§ Using suspension cultures code IV.

TABLE II Effect of elicitation using $CuSO_4 \cdot 5H_2O$ and decreasing of sucrose concentration on the formation of glucoside **3** in suspension cultures of *Solanum mammosum* (code LOG) after 7 days of incubation

Sucrose (%, w/v)	*$CuSO_4 \cdot 5H_2O$* (mg/l)	**3** (mg/g DW)*	*Biotransformation capacity* (mean ± SD, $n = 4$)	*% PCV* (mean ± SD, $n = 4$)
0	0.25	234.6 (±1.7)	72.7 ± 1.9	32.9 ± 0.7
0.25	0.25	192.4 (±1.1)	76.4 ± 3.2	47.7 ± 3.2
1	0.25	112.9 (±1.4)	65.7 ± 2.3	72.6 ± 0.2
3.0†	0.025†	77.9 (±1.1)	59.5 ± 1.5	74.5 ± 6.3

* Results are expressed as mean of duplicate analysis; % deviation from mean are given in parentheses.
† Control medium.

hormones ($n = 8$ combinations), concentrations of $CaCl_2 \cdot 2H_2O$ (110–1760 mg/l; $n = 5$) and KH_2PO_4 (170–1700 mg/l; $n = 7$) in medium, relatively did not influence the accumulation of **3** in biomass (data not shown). This work showed that elicitation using $CoCl_2 \cdot 6H_2O$ (0.05–0.5 mg/l; $n = 7$) could not stimulate the formation of **3** (data not shown), whilst elicitation using $CuSO_4 \cdot 5H_2O$ (0.125–2.5 mg/l) could increase the formation of the glucoside. The highest product was formed by elicitation using 0.25 mg/l $CuSO_4 \cdot 5H_2O$. Our experiment also showed that the difference in the initiation time of the suspension cultures (code LOG and IV) resulted in difference of biotransformation capacity. Due to its relatively high biotransformation capacity, only suspension cultures code LOG was used further.

Combining the elicitation with $CuSO_4 \cdot 5H_2O$ (0.25 mg/l) and reducing the sucrose concentration could increase the formation of **3** significantly (Table II). The maximum

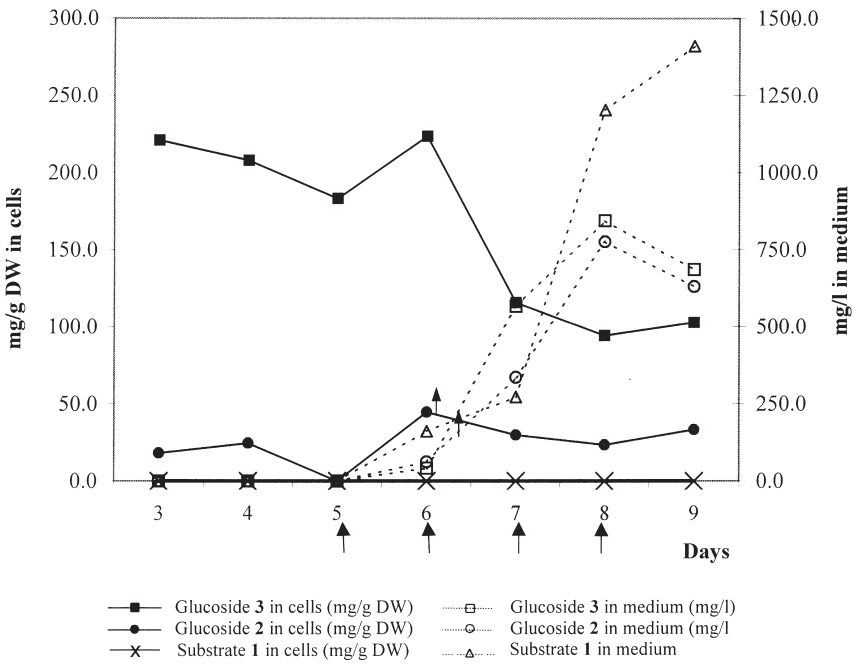

FIGURE 2 Time course of the biotransformation of **1** to **2** and **3** by cell suspension cultures of *Solanum mammosum* (code LOG) in the modified MS medium supplemented with **1** (750 mg/l), sucrose (0.25%, w/v) and $CuSO_4 \cdot 5H_2O$ (0.25 mg/l). Further addition of **1** was performed on 5–8th day (25 mg Erlenmeyer/day) and was presented by upward arrow (↑). Values represent mean of duplicate analysis (maximum deviation from mean of all data is ±5.8%).

product accumulation and biotransformation capacity were achieved in medium containing sucrose 0.25% and $CuSO_4 \cdot 5H_2O$ (0.25 mg/l). This showed that those external factors might have synergistic effect. In the medium without sucrose, packed cell volume (PCV) decreased up to 33% and the cells turned to brownish green.

To reduce its toxic effect, further addition of **1** was performed daily. Figure 2 shows that the maximum content of **3** in cells was 22.3% DW (dry weight) on the sixth day. By feeding experiment, **3** was also excreted into the medium with its maximum content (844 mg/l) on the eighth day. The mono glucoside **2** was also found in the biomass and medium. When the addition of the substrate was initiated on second day, cells were dead on the fourth day. Analysis of the cells on third day showed that the content of **3** in cells was 317.6 mg/g DW and 88.8 mg/l in medium, whilst **2** was detected only in medium (416.8 mg/l). Due to the presence of relatively high content of unbiotransformed **1** in medium (see Fig. 2, on 7–9th day), this feeding method showed low biotransformation capacity (*ca.* 40%), so through further optimization of feeding method and cell selection, we believe that the formation of the product can be enhanced.

This work demonstrated that cell suspension cultures of *Solanum mammosum* can be used for producing the glucosides of **1** in relatively high yield. In this work, accumulation of the product **3** in relatively high yield (*ca.* 20–30% DW) was achieved.

Acknowledgements

This work was financially supported by the Ministry of Research and Technology (RUT VI/3, 2000) and the Ministry of National Education of the Republic of Indonesia (URGE-S2-Grant for LH). The technical assistance of O. Yunita, A.T. Purnomo and A. Rahman were greatly appreciated.

References

[1] Suga, T. and Hirata, T. (1990), *Phytochemistry* **29**, 2393–2406.
[2] Dombrowski, K. (1993) "Phytochemische und enzymologische Untersuchungen zur Biotransformation von Salicylverbindungen durch Zellkulturen der Weidenart Salix matsudana f. tortousa" Dissertation (Universität Düsseldorf, Germany).
[3] Inomata, S., Yokoyama, M., Seto, S. and Yanagi, M. (1991), *Appl. Microbiol. Biotechnol.* **36**, 315–319.
[4] Lutterbach, L. and Stöckigt, J. (1992), *Helv. Chim. Acta* **75**, 2009–2011.
[5] Syahrani, A., Indrayanto, G. Wilkins, A. and Sutarjadi (1997) *Nat. Prod. Sci.* **3**, 71–74.
[6] Syahrani, A., Indrayanto, G., Wilkins, A. and Sutarjadi (1997), *Chem. Pharm. Bull.* **45**, 555–557.
[7] Syahrani, A., Ratnasari, E., Indrayanto, G. and Wilkins, A.L. (1999), *Phytochemistry* **51**, 615–620.
[8] Parfitt, K. (1999) Martindale, the Complete Drug Reference (Pharmaceutical Press, London), p. 1486.
[9] Margalin, H. (1999) "Biotransformasi asam orto aminobenzoat dengan kultur suspensi Solanum mammosum" BSc Thesis (Faculty of Pharmacy, Airlangga University).
[10] Indrayanto, G., Erawati, T. and Santosa, M.H. (1995), *Plant Cell Tiss. Org. Cult.* **43**, 237–240.
[11] Murashige, T. and Skoog, F. (1962), *Physiol. Plant.* **15**, 473–479.
[12] Seitz, H.U., Seitz, U. and Alfermann, W. (1985) Pflanzliche Gewebekultur (Gustav Fisher Verlag, Stuttgart, NY), pp. 78–79.
[13] Indrayanto, G., Margalin, H., Ratnasari, E. and Syahrani, A. (1999), *J. Planar. Chromatogr.* **12**, 456–460.