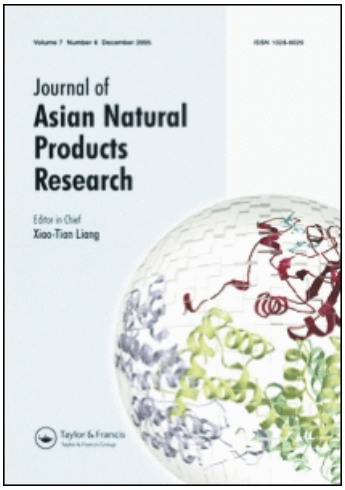

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DIGLUCOSYLATION OF SALICYL ALCOHOL BY CELL SUSPENSION CULTURES OF *SOLANUM LACINIATUM*

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A new biotransformation product, salicyl alcohol-7-O-β-D-(β-1,6-D-glucopyranosyl)-glucopyranoside was isolated from cell suspension cultures of *Solanum laciniatum*, following administration of salicyl alcohol, and its structure was elucidated using a combination of one- and two-dimensional ^{1}H and ^{13}C-NMR data, and positive and negative ion ESMS data.

Keywords: *Solanum laciniatum*; Cell suspension cultures; Biotransformation; Glucosylation; Salicyl alcohol; Salicyl alcohol-7-O-β-D-(β-1,6-D-glucopyranosyl)-glucopyranoside

INTRODUCTION

Various plant cell cultures are capable of glucosylating a variety of exogenously supplied substrates [1]. The glucosylation of simple phenols by various cell suspension cultures have been reported by Umetani, Tanaka and Tabata [2]. 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 [3]. We have previously reported the bioconversion of

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salicyl alcohol into salicin, salicylamide into salicylamide 2-O-β-D-glucopyranoside, *p*-aminobenzoic acid into *p*-aminobenzoic acid-7-O-β-D-glucopyranosyl ester, and *o*-aminobenzoic acid into *o*-aminobenzoic acid-7-O-β-D-glucopyranosyl ester and *o*-aminobenzoic acid-7-O-β-D-(β-D-1,6-O-glucopyranosyl)-glucopyranosyl ester by cell suspension cultures of *Solanum mammosum* [4–6]. We have also found that cell suspension cultures of *Solanum laciniatum* transformed inoculated salicyl alcohol into salicyl alcohol-7-O-β-D-glucopyranoside (isosalicin) [7].

We now report the conversion of salicyl alcohol (**1**) by cell suspension cultures of *Solanum laciniatum* into a new biotransformation product, salicyl alcohol-7-O-β-D-(β-1,6-D-glucopyranosyl)-glucopyranoside (**2**) and its structure elucidation, using a combination of one- and two-dimensional ^{1}H and ^{13}C-NMR, and positive and negative ion ESMS data.

RESULTS AND DISCUSSION

Incubation of cell suspension cultures of *Solanum laciniatum* with **1** (750 mg l^{-1}), followed by isolation, purification by column chromatography and preparative TLC, afforded a major metabolite (compound **3**) and a minor metabolite (compound **2**). TLC analysis showed that the spots of substrate **1** ($R_f = 0.77$), metabolite **2** ($R_f = 0.13$) and metabolite **3** ($R_f = 0.40$), exhibited very similar *in situ* UV absorbance reflectance spectra. Control experiments showed that in the absence of cells, substrate **1** remaines unchanged in the culture medium and that metabolites **2** and **3** were only produced when cell suspension cultures of *Solanum laciniatum* were present in the medium. ^{1}H and ^{13}C-NMR analysis showed that **3**, the major metabolite, was the known 7-O-β-D-glucopyranoside analogue of **1** (isosalicin) [7].

The positive ion electrospray mass spectrum (ESMS) of **2**, determined in a cation assisted matrix, included pseudomolecular ions at m/z 466 $(M+NH_4)^+$, 471 $(M+Na)^+$, 487 $(M+K)^+$, 914 $(2M+NH_4)^+$ and 934 $(2M+K)^+$. A pseudomolecular ion also appeared at m/z 447 $(M-H)^-$ in the negative ion ESMS of this metabolite. These ESMS data indicated that metabolite **2** has a molecular weight of 448 daltons, and that it is a diglucoside analogue of **1**. The ^{13}C-NMR spectrum of **2** was comprised of 3 methylene, 14 methine and 2 quaternary resonances (see Tab. I). The ^{13}C-NMR spectrum of the minor metabolite **2** was similar to that of the major metabolite **3**, except for the occurrence of the six additional signals, typical of a glucopyranosyl residue. The ^{1}H-NMR spectrum of **2** included signals at δ 4.34 (H-1′, *d*, $J = 7.8$ Hz) and 4.36 (H-1″, *d*, $J = 7.8$ Hz) attributable

TABLE I. ^{1}H-and ^{13}C-NMR spectral data (in DMSO-d_6) of metabolites **2** and **3**[a]

	Metabolite **3**[b]		*Metabolite* **2**	
	^{13}C	^1H	^{13}C	^1H
C-1	124.4	–	124.3	–
C-2	154.5	9.45 (br.*s*, **OH**)	154.8	9.50 (br.*s*, **OH**)
C-3	114.8	6.86 (*m*)	114.9	6.87 (*d*, $J=7.6$)
C-4	128.1	7.16 (*ddd*, $J=1.8$, 7.4, 8.0)	128.3	7.16 (*t*, $J=7.5$)
C-5	118.7	6.85 (*m*)	118.7	6.84 (*t*, $J=7.4$)
C-6	128.6	7.45 (*dd*, $J=1.7$, 7.5)	129.1	7.44 (*d*, $J=7.3$)
C-7	65.0	4.64 (*d*, $J=12.9$)	65.3	4.62 (*d*, $J=12.6$)
		4.86 (*d*, $J=12.9$)		4.86 (*d*, $J=12.6$)
C-1′	102.4	4.33 (*d*, $J=7.7$)	102.4	4.34 (*d*, $J=7.8$)
C-2′	73.6	3.12 (*m*, H_{ax})	73.6	3.11 (*m*, H_{ax})
		5.12 (br.*d*, $J=4.6$, **OH**)		5.12 (br.*s*, **OH**)
C-3′	76.8	3.22 (*m*, H_{ax})	76.7	3.23 (*m*, H_{ax})
		4.98 (br.*d*, $J=4.6$, **OH**)		4.99 (br.*s*, **OH**)
C-4′	70.2	3.18 (*m*, H_{ax})	70.0	3.18 (*m*, H_{ax})
		4.95 (br.*d*, $J=4.8$, **OH**)		4.99 (br.*s*, **OH**)
C-5′	76.9	3.19 (*m*, H_{ax})	76.9	3.18 (*m*, H_{ax})
C-6′	61.0	3.55 (*ddd*, $J=5.7$, 5.7, 11.7)	68.3	3.70 (*m*)
		3.78 (*ddd*, $J=1.9$, 5.7, 11.7)		4.09 (*m*)
		4.58 (br.*t*, $J=5.8$, **OH**)		
C-1″			103.1	4.36 (*d*, $J=7.8$)
C-2″			73.5	3.14 (*m*, H_{ax})
				4.99 (br.*s*, **OH**)
C-3″			75.9	3.44 (*m*, H_{ax})
				5.19 (br.*s*, **OH**)
C-4″			69.9	3.21 (*m*, H_{ax})
				5.12 (br.*s*, **OH**)
C-5″			76.7	3.18 (*m*, H_{ax})
C-6″			61.1	3.53 (*m*)
				3.75 (*m*)
				4.60 (br.*s*, **OH**)

[a] δ in ppm and J (parantheses) in Hz.
[b] Cited from Syahrani *et al.* [7].

to the presence of two anomeric β-glycopyranosyl protons. Four aryl proton signals were also observed in the ^{1}H-NMR spectrum of **2** (see Tab. I).

The HMBC spectrum of **2** included correlations between the anomeric glucosyl protons at δ 4.34 (H-1′) with C-7 (δ 65.3) and δ 4.36 (H-1″) with C-6′ (δ 68.3), while H-7_A/7_B (δ 4.62 and 4.86) exhibited correlations with C-1′ (δ 102.4), C-1 (δ 124.3), C-6 (δ 129.1) and C-2 (δ 154.8). These data indicated that the inner β-D-glucopyranosyl residue was attached to C-7, and that the pair of the β-D-glucopyranosyl residues were mutually 1,6-linked. Comparison with the ^{13}C-NMR assignments determined for the glucosyl carbons of **3** showed that the C-6′ resonance of **2** exhibited a downfield shift of 7.3 ppm, indicative of α-glucosylation [8]. Correlations observed in the

ROESY spectrum of **2** also established the presence of a 1,6-glucosidic linkage. In particular, correlations were observed between H-1′ (4.34 ppm) and H-7_A/7_B (4.62 and 4.86 ppm) and between H-1″ (4.36 ppm) and H-$6'_A$/$6'_B$ (3.70 and 4.09 ppm). Accordingly, the structure of **2** was determined to be salicyl alcohol-7-O-β-D-(β-1,6-D-glucopyranosyl)-glucopyranoside.

To our knowledge this is the first report of the diglucosylation of salicyl alcohol by cell suspension cultures of *Solanum laciniatum*. This also appears to be the first report of the isolation of salicyl alcohol-7-O-β-D-(β-1,6-D-glucopyranosyl)-glucopyranoside (**2**) from natural sources. It seems likely that cell suspension cultures of *Solanum laciniatum* first glucosylate **3** at C-7, prior to glucosylation at C-6′ to form **2**.

EXPERIMENTAL SECTION

General Experimental Procedures

NMR spectra were determined at 400.13 MHz (^{1}H) and 100.62 (^{13}C) using an inverse 5 mm probe head installed in a Bruker DRX 400 spectrometer. Gradient selection was utilized in HMBC and HSQC experiments. Chemical shift (δ ppm) are reported relative to solvent peaks observed for DMSO-d_6 (^{1}H = 2.60 ppm; ^{13}C = 39.5 ppm). Coupling constants are reported to a precision of ± 0.2 Hz. ^{13}C-NMR signal multiplicities (*d*, *t* or *q*; *s* suppressed) were determined using the distortionless enhancement by polarization transfer (DEPT) sequence with a 135° detection pulse. Two-dimensional COSY and HMBC (80 msec mixing time) spectra were determined in absolute value mode, while TOCSY, ROESY (250 msec spin lock time) and HSQC spectra were determined in phase sensitive mode.

The positive and negative ion electrospray mass spectra (ESMS) were determined using a Fisons VG Platform II instrument. Samples were introduced into the spectrometer using CH_3CN/H_2O 1:1 as solvent.

The *in situ* UV absorbance reflectance spectra of the TLC spots of substrate **1**, and metabolites **2** and **3**, were determined using a Shimadzu TLC Scanner CS 930. Stationary phase: silica gel F254 precoated plate (Merck); mobile phase: EtOAc/MeOH/H_2O 77 : 13 : 10.

Cell Suspension Culture and Biotransformation Condition

Cell suspension cultures were initiated from callus cultures of *Solanum laciniatum* Ait. (code sl-4), as previously reported [7]. The calli were

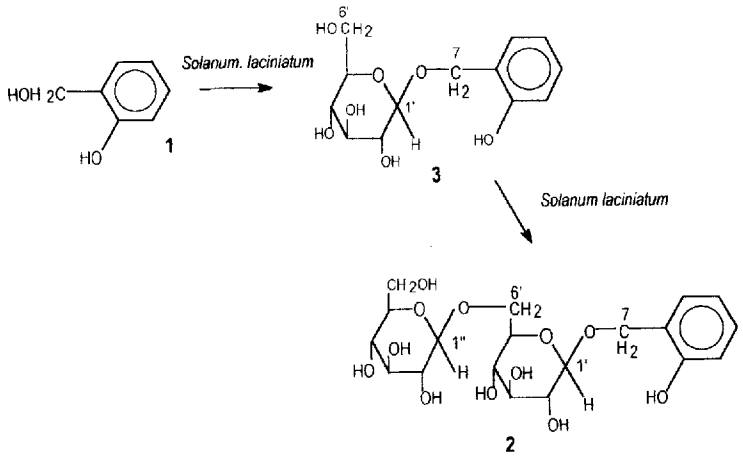

FIGURE 1 The biotransformation of substrate **1** to metabolites **3** and **2** by cell suspension cultures of *Solanum laciniatum*.

cultivated in 300 ml Erlenmeyer flasks containing 50 ml of modified Murashige and Skoog medium [9] supplemented with sucrose ($30\,g\,l^{-1}$), kinetin ($2\,mg\,l^{-1}$), NAA ($1\,mg\,l^{-1}$) and casein hydrolisate ($1\,g\,l^{-1}$) on a gyrotary shaker (100 rpm) at $25 \pm 1°$ under continuous light (ca. 1500 lux).

Biotransformation experiments were typically performed by inoculating cells (10 g fresh weight) into the liquid medium (50 ml) containing **1** ($750\,mg\,l^{-1}$) and incubated for 7 days. After 7 days the cultures were harvested, filtered, weighed, oven dried at 40°C (until their water content was ca. 2%), and powdered [7].

Biotransformation of 1 and Isolation of 2 and 3

The oven dried (40°C) powdered biomass (18.90 g) collected from 40 Erlenmeyer flasks was refluxed (2 h) in MeOH. The MeOH extract was concentrated under reduction pressure to afford a dark brown semi solid residue (8.34 g), which was submitted to column chromatography on silica gel 40 (70 – 230 Mesh ASTM) (Merck) using EtOAc/MeOH/H_2O 77 : 13 : 10 as eluent. Purification of the glucoside fractions by preparative TLC (precoated silica gel 60 GF 254; Merck) using EtOAc/MeOH/H_2O 77 : 13 : 10 as developing solvent, yielded **2** (18.5 mg) and isosalicin (**3**) (719 mg) [7].

Metabolite **2**, a white amorphous solid; *in situ* UV absorbance reflectance, λ_{max} nm (silica gel F_{254}): 273; positive ion ESMS (+60V, Na^+, K^+ and

NH_4^+ assisted matrix), *m/z* (% rel.int): 466 ($[M+NH_4]^+$, 81), 471 ($[M+Na]^+$, 94), 487 ($[M+K]^+$, 16), 914 ($[2M-NH_4]^-$, 10), 934 ($[2M+K]^+$, 3); negative ion ESMS (−60 V), *m/z* (%rel. int): 447 ($[M-H]^-$, 87); ^{13}C- NMR and ^1H-NMR see Table I.

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