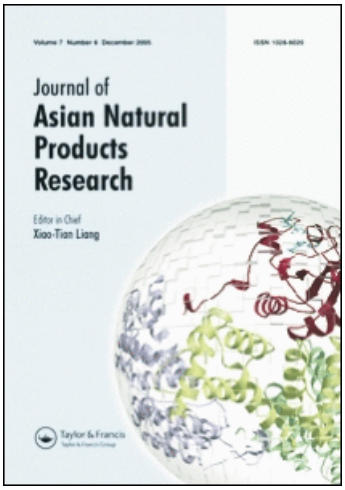

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N-ACETYLATION AND *N*-FORMYLATION OF *m*-AMINOBENZOIC ACID BY CELL SUSPENSION CULTURES OF *SOLANUM LACINIATUM*

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Two new biotransformation products, *N*-acetyl-*m*-aminobenzoic acid and *N*-formyl-*m*-aminobenzoic acid were isolated from cell suspension cultures of *Solanum laciniatum* following administration of *m*-aminobenzoic acid, and their structures were elucidated using one- and two-dimensional ^{1}H- and ^{13}C-NMR data.

Keywords: *Solanum laciniatum*; Cell suspension cultures; Biotransformation; *N*-Acetylation; *N*-Formylation; *N*-Acetyl-*m*-aminobenzoic acid; *N*-Formyl-*m*-aminobenzoic acid; *m*-Aminobenzoic acid

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 Umetami *et al.* [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].

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We have previously reported the glucosylation of salicyl alcohol into salicin [4], salicylamide into salicylamide 2-O-β-D-glucopyranoside [5], *p*-aminobenzoic acid into *p*-aminobenzoic acid-7-O-β-D-glucopyranosyl ester, *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* [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] and salicyl alcohol-7-O-β-D-(β-1,6-D-glucopyranosyl)-glucopyranoside [8]. Interestingly, the cell suspension cultures of *Solanum mammosum* could also transform *p*-aminobenzoic acid into its *N*-acetyl and *N*-formyl analogues [6].

Now we are reporting the conversion of *m*-aminobenzoic acid (**1**) by cell suspension cultures of *Solanum laciniatum* into two new biotransformation products, *N*-acetyl-*m*-aminobenzoic acid (**2**), *N*-formyl-*m*-aminobenzoic acid (**3**) and its structure elucidation, using a combination of one- and two-dimensional ^{1}H- and ^{13}C-NMR.

RESULTS AND DISCUSSION

Incubation of the cell suspension cultures prepared as reported previously [7] with **1** followed by isolation (column chromatography and preparative TLC), gave a metabolite spot on TLC plate (R_f 0.49). Control experiments showed in the absence of cells, **1** (R_f 0.74) remained unchanged in the medium, and the metabolites were only formed when **1** was added in the suspension cultures.

One- and two-dimensional NMR analyses of the isolated metabolite material showed that it was *ca.* 6:1 mixture of *N*-acetyl-*m*-aminobenzoic acid (**2**) and *N*-formyl-*m*-aminobenzoic acid (**3**). The HMBC spectrum of the mixture showed correlation between the amide proton (–N**H**) of **2** (10.04 ppm) and C-3 (138.4 ppm), C-2 (120.3 ppm) and C-8 (168.2 ppm), and between H-6 (7.62 ppm) and C-2 (120.3 ppm), C-4 (119.8 ppm), and C-7 (169.8 ppm). Correlation of H-8 (8.32 ppm) of **3** with C-3 (137.4 ppm) was also observed. Both metabolites showed HMBC correlations between H-2 and C-4, C-6 and C-7 and between H-4 with C-2, C-3 and C-6. Correlations were also observed in the ROESY spectrum between the –N**H** proton of **2** and H-2 (8.04 ppm), H-4 (7.74 ppm) and –C**H$_3$** (H-9, 2.11 ppm).

To our knowledge, this is the first report of the biotransformation of **1** by suspension cultures of *Solanum laciniatum* (see Fig. 1). This also appears to

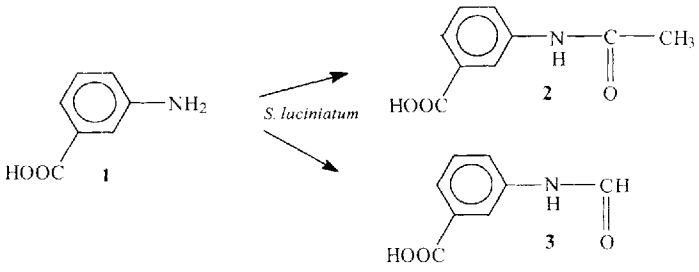

FIGURE 1 The biotransformation of **1** into **2** and **3** by cell suspension cultures of *Solanum laciniatum*.

be the first report of the isolation of *N*-acetyl-*m*-aminobenzoic acid (**2**) and *N*-formyl-*m*-aminobenzoic acid (**3**) from natural sources. While the ability of various cell suspension cultures to perform various biotransformation reactions (glycosylation, hydroxylation, oxidation, reduction, etc.) of exogenously supplied substrates is well known [1], *N*-acetylation and *N*-formylation have, to date, only been observed for *Solanum laciniatum* and *Solanum mammosum* [6] suspension cultures. It is yet to be known if *N*-acetylation and *N*-formylation is a more general phenomena in this genus, or this capability is confined to certain substrates. Although suspension cultures of *Solanum mammosum* [4–6] and *Solanum laciniatum* [7,8] could transform some other similar substrates into their glycosides analogue, the presence of the glycoside of **1** could not be detected in this work.

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 $\pm$0.2 Hz. ^{13}C-NMR signal multiplicity's (*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 ms mixing time) spectra were determined in absolute value mode, while TOCSY, ROESY (250 ms spin lock time) and HSQC spectra were determined in phase sensitive mode.

The TLC analyses were performed on silica gel F_{254} precoated plate (E. Merck); solvent system; EtOAc/MeOH/H_2O 77 : 13 : 10.

Cell Suspension Culture and Biotransformation Conditions

Cell suspension cultures were initiated from callus cultures of *Solanum laciniatum* Ait. (code sl-7), as previously reported [7]. The calli were 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 (10.57 g) collected from 20 Erlenmeyer flasks was refluxed (2 h) in MeOH three times. The MeOH extract was concentrated under reduced pressure to afford a dark brown semi solid residue (2.90 g), which was submitted to column chromatography on silica gel 40 (70–230 Mesh ASTM) (E. Merck) using EtOAc/MeOH/H_2O 77 : 13 : 10 as eluent. Purification of the metabolite fractions by preparative TLC (precoated silica gel 60 GF 254; E. Merck) (solvent system EtOAc/MeOH/H_2O 77 : 13 : 10), yielded a mixture of **2** and **3** (16.0 mg).

Metabolite 2

^{1}H-NMR (DMSO-d_6, 400.13 MHz): $\delta = 10.04$ (s, 1H, –NH), 8.04 (s, 1H, H-2), 7.74 (d, 1H, 7.8 Hz, H-4), 7.62 (d, 1H, 7.5 Hz, H-6), 7.25 (m, 1H, H-5), 2.11 (s, 3H, $-COCH_3$); ^{13}C-NMR (DMSO-d_6, 100.6 MHz): $\delta = 140.7$ (C-1), 120.3 (C-2), 138.4 (C-3), 119.8 (C-4), 127.3 (C-5), 124.0 (C-6), 169.8 (C-7), 168.2 (C-8), 24.0 (C-9).

Metabolite 3

^{1}H-NMR (DMSO-d_6, 400.13 MHz): $\delta = 10.35$ (br, s, 1H, –NH), 8.32 (d, 1H, 1.6 Hz, H-8), 8.06 (s, 1H, H-2), 7.74 (d, 1H, 7.8 Hz, H-4), 7.68 (d, 1H, 7.2 Hz, H-6), 7.29 (m, 1H, H-5). ^{13}C-NMR (DMSO-d_6, 100.6 MHz): $\delta = 141.0$ (C-1),

120.3 (C-2), 137.4 (C-3), 119.9 (C-4), 127.5 (C-5), 124.6 (C-6), 169.6 (C-7), 159.5 (C-8).

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