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Biotransformation of *o*- and *p*-aminobenzoic acids and *N*-acetyl *p*-aminobenzoic acid by cell suspension cultures of *Solanum mammosum*

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Abstract

Some new biotransformation products, *p*-aminobenzoic acid 7-*O*-β-D-glucopyranosyl ester, *N*-acetyl *p*-aminobenzoic acid 7-*O*-β-D-glucopyranosyl ester, *o*-aminobenzoic acid 7-*O*-β-D-(β-1,6-*O*-D-glucopyranosyl)glucopyranosyl ester and *o*-aminobenzoic acid 7-*O*-β-D-glucopyranosyl ester were isolated from cell suspension cultures of *Solanum mammosum* following administration of *p*-aminobenzoic acid, *N*-acetyl *p*-aminobenzoic acid or *o*-aminobenzoic acid respectively. *N*-acetyl *p*-aminobenzoic acid and *N*-formyl *p*-aminobenzoic acid were also identified as cell suspension metabolites of *p*-aminobenzoic acid. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Solanum mammosum; Cell suspension culture; Biotransformation; *p*-Aminobenzoic acid 7-*O*-β-D-glucopyranosyl ester; *N*-acetyl *p*-aminobenzoic acid 7-*O*-β-D-glucopyranosyl ester; *N*-acetyl *p*-aminobenzoic acid; *N*-formyl *p*-aminobenzoic acid; *o*-Aminobenzoic acid 7-*O*-β-D-glucopyranosyl ester; *o*-Aminobenzoic acid 7-*O*-β-D-(β-1,6-*O*-D-glucopyranosyl)glucopyranosyl ester

1. Introduction

Biological transformation (biotransformation) using plant cell suspension cultures serves as an important tool in the structural modification of compounds possessing useful therapeutic activity (Kamel, Brazier, Desmet, Flinaux, & Dubreuil, 1992). Various plant cell cultures are capable of glycosylating a variety of exogenously supplied compounds (Suga & Hirata, 1990). The glycosylation of simple phenols (e.g. salicyl alcohol and salicylic acid) by various cell suspension cultures have been reported by Umetami, Tanaka, and Tabata (1982). The glucosylation of isomeric hydroxybenzoic acids by cell suspension cultures of *Mallotus japonicus* has been reported by Tanaka, Hayakawa, Umetami, and Tabata (1990), while Mizukami, Terao, Miura, and Ohashi (1983); Mizukami, Terao, and Ohashi (1986) have reported that some cell suspension cultures (e.g., *Lithospermum erythrorhizon, Gardenia jasminoides*) were able to transform salicylate derivatives, such as salicyl alcohol, into salicin and isosalicin. Dombrowski and Alfermann (1992) and Dombrowski (1993) have reported the transformation of salicyl alcohol into salicin and isosalicin, salicylic acid into salicylic acid 2-*O*-β-D-glucopyranoside and salicylic acid 7-*O*-β-D-glucopyranosyl ester in the suspension cultures of *Salix matsudana*. In previous papers (Syahrani, Indrayanto, Sutarjadi, & Wilkins, 1997b; Syahrani, Indrayanto, Wilkins & Sutarjadi, 1997a), we have reported that cultured suspension cells of *Solanum mammosum* were able to transform salicylamide into salicylamide 2-*O*-β-D-glucopyranoside and salicyl alcohol into salicyl alcohol 2-*O*-β-D-glucopyranoside.

We are now reporting the formation and structure elucidation of *p*-aminobenzoic acid 7-*O*-β-D-glucopyranosyl ester (**1**), *N*-acetyl *p*-aminobenzoic acid (**2**), *N*-

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formyl *p*-aminobenzoic acid (**3**) from exogenously supplied *p*-aminobenzoic acid (**7**); *N*-acetyl *p*-aminobenzoic acid 7-*O*-β-D-glucopyranosyl ester (**4**) from *N*-acetyl *p*-aminobenzoic acid (**2**); and *o*-aminobenzoic acid 7-*O*-β-D-(β-1,6-*O*-D-glucopyranosyl) glucopyranosyl ester (**5**), *o*-aminobenzoic acid 7-*O*-β-D-glucopyranosyl ester (**6**) from *o*-aminobenzoic acid (**8**) in suspension cultures of *S. mammosum*.

2. Results and discussion

Toxicity of **2**, **7** and **8** toward cell suspension cultures of *S. mammosum* was investigated for concentrations in the range of 500–1500 mg l^{-1}. Cell death occurred at concentrations >1250 mg l^{-1} for **2** and **7** and 1000 mg l^{-1} for **8**. At concentrations of 1000 mg l^{-1} for (**2**) and (**7**) and 750 mg l^{-1} for (**8**), cells survived, but did not grow (packed cell volumes — PCV — remains relatively constant). Toxicity of **2** and **7** are equivalent to the toxicity of salicylamide and salicyl alcohol as previously reported (Syahrani et al., 1997b; Syahrani et al., 1997a), whilst **8** appeared to be more toxic toward this suspension cultures.

Incubation of cell suspension cultures of *S. mammosum* — prepared, as reported previously (Syahrani et al., 1997b; Syahrani et al., 1997a) — with *p*-aminobenzoic acid (**7**) followed by isolation, purification by column chromatography and preparative TLC, gave metabolite **1** (R_{f} 0.37) and mixture of metabolites **2** and **3** (R_{f} 0.51); incubation of *N*-acetyl *p*-aminobenzoic acid (**2**) produced metabolite **4** (R_{f} 0.25). Metabolites **5** (R_{f} 0.08) and **6** (R_{f} 0.42) were obtained by incubation of the cultures with *o*-aminobenzoic acid (**8**). Control experiments showed that in the absence of cells, substrates **7** (R_{f} 0.81), **2** (R_{f} 0.51) and **8** (R_{f} 0.82) remain unchanged in the culture medium and that metabolites **1**, **2**, **3**, **4**, **5** or **6** were only produced when cell suspension cultures of *S. mammosum* were present in the medium.

Incubation with *p*-aminobenzoic acid (**7**) afforded metabolite (**1**) which exhibited ^{1}H and ^{13}C NMR signals (Table 1) that indicated it was a glucosyl analogue of (**7**). The positive ion electrospray mass spectrum (ESMS) of **1**, determined in a Na^{+} and NH_4^{+} assisted matrix, included pseudomolecular ions at *m/z* 300 $(M+H)^{+}$, 317 $(M+NH_4)^{+}$ and 322 $(M+Na)^{+}$, and cluster ions at *m/z* 616 $(2\ M+NH_4)^{+}$ and 621 $(2\ M+Na)^{+}$. The negative ion ESMS, determined in a Cl^{-} assisted matrix, included pseudomolecular ions at *m/z* 298 $(M–H)^{-}$, 334 $(M+{}^{35}Cl)^{-}$ and 336 $(M+{}^{37}Cl)^{-}$, and cluster ions at *m/z* 597 $(2\ M–H)^{-}$, 633 $(M+{}^{35}Cl)^{-}$ and 635 $(M+{}^{37}Cl)^{-}$. One- and two-dimensional NMR spectral data (including COSY, ROESY, HSQC and HMBC spectra) identified **1** as *p*-aminobenzoic acid 7-*O*-β-D-glucopyranosyl ester. The site of attachment of the glucopyranosyl unit was elucidated from a HMBC correlation between H-1′ (5.59 ppm) and C-7 (164.6 ppm), while the β-orientation of the glucoside linkage was defined by the coupling constant (d, $J=7.8$ Hz) determined for H-1′ and by correlations between H-1′, and H-3′ (3.25 ppm) and H-5′ (3.39 ppm) respectively, observed in the ROESY spectrum of **1**.

Table 1
^{13}C NMR chemical shifts (δ DMSO-d_6) of some cell culture β-D-glucopyranosides and β-D-glucopyranosyl esters

Atom	**1**	**4**	**5**	**6**
1	115.1	132.2	107.9	108.1
2	131.6	130.7	151.8	151.8
3	112.7	118.2	116.5	116.5
4	153.9	144.0	134.5	134.5
5	112.7	118.2	114.6	114.7
6	131.6	130.7	131.0	131.0
7	164.6	164.2	166.0	166.1
8		169.0		
9		24.1		
1′	94.2	94.7	94.1	94.2
2′	72.6	72.5	72.4	72.5
3′	76.6	76.4	76.4	76.5
4′	69.6	69.5	69.3	69.8
5′	77.8	77.9	76.3	77.9
6′	60.6	60.6	68.0	60.6
1″			103.1	
2″			73.4	
3″			76.7	
4″			69.9	
5″			76.8	
6″			60.9	

One- and two-dimensional NMR analyses of a minor fraction recovered after incubation with *p*-aminobenzoic acid revealed it to be a ca 3:1 mixture of *N*-acetyl *p*-aminobenzoic acid (**2**) and *N*-formyl *p*-aminobenzoic acid (**3**). The HMBC spectrum of the mixture showed correlations between the amide proton (-NH) (10.09 ppm) of *2* and C-3 (=C-5) (117.5 ppm) and C-8 (168.2 ppm), and between H-2 (7.88 ppm) and C-7 (169.7 ppm), C-3 (117.5 ppm), C-4 (139.8 ppm) and C-6 (129.5 ppm). Correlations were observed in the ROESY spectrum between -NH proton of **2** and H-3 (=H-5) (7.57 ppm) and H-9 (2.12 ppm). The HMBC spectrum of the mixture also included correlations between the amide proton (-NH) (10.42 ppm) of **3** and C-3 (=C-5) (117.7 ppm) and between the formyl proton (H-8) (8.34 ppm) of **3** and C-4 (138.7 ppm). Correlations were also observed in the ROESY spectrum between the -NH proton of **3** and H-3 (7.57 ppm) and H-8 (8.34 ppm). A synthetic specimen of *N*-acetyl *p*-aminobenzoic (**2**), subsequently utilized as a substrate in a cell experiment, exhibited ^{1}H and ^{13}C NMR resonances identical to those attributable to the presence of **2** in the mixture of **2** and **3**. GC/MS analyses of a methylated subsample of the mixture of **2** and **3** afforded peaks assigned to methyl esters of **2** (compound **9**) [m/z 193 (M^+)] and methyl ester of **3** (compound **10**) [m/z 179 (M^+)], respectively.

The ^{13}C NMR spectrum of metabolite **4** (Table 1), isolated after incubation of *N*-acetyl *p*-aminobenzoic acid (**7**) indicated it to be a glucopyranosyl analogue of **7**. The ^{1}H NMR spectrum of the metabolite included signals attributable to an anomeric β-D-glucopyranosyl proton (5.63 ppm, $J=7.7$ Hz) (Agrawal, 1992), an amide proton (10.37 ppm) and an *N*-acetyl group (2.16 ppm), while the HMBC spectrum included correlations between H-1′ (5.63 ppm) and C-7 (164.2 ppm); H-9 (2.16 ppm) and C-8 (169.0 ppm); and H-2 (=H-6) (8.03 ppm) and C-7 (164.2 ppm). These data differentiated the ester and amide carbonyl resonances (164.2 and 169.0 ppm respectively) and identified **4** as *N*-acetyl *p*-aminobenzoic acid 7-*O*-β-D-glucopyranosyl ester.

The ^{13}C NMR spectrum of the major metabolite **5**, isolated after incubation of *o*-aminobenzoic acid (**8**), was comprised of 2 methylene, 14 methine and 3 quaternary resonances (see Table 1). The ^{1}H-NMR spectrum of **5** included 4 aryl proton signals, two doublet resonances centered at 5.61 and 4.25 ppm ($J=7.9$ Hz and $J=7.7$ Hz respectively) assigned to the anomeric protons of two β-D-glucopyranosyl residues and a broad two proton -NH_2 signal (6.74 ppm). These data are consistent with the formulation of **5** as a diglucopyranosyl analogue of **8**. HMBC spectral data established that the inner glucopyranosyl residue was attached to the carboxyl group of the substrate and that the pair of β-D-glucopyranosyl residues were mutually 1,6-linked. In particular, HMBC correlations were observed between H-1′ (5.61 ppm) and C-7 (166.0 ppm), and between H-1″ (4.25 ppm) and C-6′ (68.0 ppm). A notable feature of the ^{13}C NMR spectrum of **5** is downfield shift of the C-6′ signal (68.0 ppm) in comparison to the resonance of the C-6″ signal (60.9 ppm). This downfield shift is consistent with C-6′ glucosylation (Agrawal, 1992). Correlations observed in the ROESY spectrum of **5** between the H-1″ (4.25 ppm) resonance of the outer glucopyranosyl unit and the H-$6'_A$ and the H-$6'_B$ (3.68 and 4.08 ppm respectively) resonances of the inner glucopyranosyl unit, and mutual correlations between the H-1′, H-3′ and H-5′, and the H-1″, H-3″ and H-5″ resonances of the inner and outer glucopyranosyl residues respectively, were consistent with the identification of **5** as *o*-aminobenzoic acid 7-*O*-β-D-(β-1,6-*O*-D-glucopyranosyl) glucopyranosyl ester.

ESMS data was also consistent to the identification of **5** as a diglucopyranosyl ester. In particular, the positive ion ESMS spectrum of **5**, determined in Na^+ assisted matrix, included ions attributable to $(M+H)^+$ and $(M+Na)^+$ pseudomolecular ions (m/z 462 and 484 respectively), together with $(2\ M+H)^+$ and $(2\ M+Na)^+$ cluster ions (m/z 923 and 945 respectively), while the negative ion ESMS of **5**, determined in a Cl^- assisted matrix, included ions identified as $(M–H)^-$, $(M+{}^{35}Cl)^-$ and $(M+{}^{37}Cl)^-$ pseudomolecular ions (m/z 460, 496 and 498 respectively).

One- and two-dimensional NMR and electrospray mass spectral data identified the minor metabolite recovered after incubation of *o*-aminobenzoic (**8**) as *o*-amino benzoic acid 7-*O*-β-D-glucopyranosyl ester (**6**). The ^{13}C NMR spectrum of **6** was comprised of a methylene, 9 methine and 3 quaternary resonances (see Table 1), while the ^{1}H NMR spectrum included signals assigned to an anomeric β-D-glucopyranosyl proton (H-1′) (5.61 ppm, $J=7.9$ Hz) and two amine (-NH_2) protons (6.73 ppm). The HMBC spectrum of **6** included correlation between H-1′ (5.61 ppm) and C-7 (166.1 ppm), H-6 (7.86 ppm) and C-2 (151.8 ppm), C-4 (134.5 ppm) and C-7 (166.1 ppm) and the amine protons (-NH_2) (6.73 ppm) and C-1 (108.1 ppm) and C-3 (116.5 ppm). The positive ion ESMS of **6**, determined in Na^+ assisted matrix, showed ions of $(M+H)^+$ and $(M+Na)^+$ pseudomolecular ions (m/z 300 and 322 respectively), together with $(2\ M+H)^+$ and $(2\ M+Na)^+$ cluster ions (m/z 599 and 621 respectively). The negative ion ESMS of **6**, determined in a Cl^- assisted matrix, showed peaks of $(M–H)^-$, $(M+{}^{35}Cl)^-$ and $(M+{}^{37}Cl)^-$ pseudomolecular ions (m/z 298, 334 and 336 respectively), together with $(2\ M–H)^-$, $(2\ M+{}^{35}Cl)^-$ and $(2\ M+{}^{37}Cl)^-$ cluster ions (m/z 597, 633 and 635 respectively).

3. Conclusions

To our knowledge this is the first report of the biotransformation of **2**, **7** and **8** to the β-D-glucopyranosyl esters (**1**, **4**, **5**, or **6**), by cell suspension cultures of *S. mammosum*. None of these glucopyranosyl esters appear to have been previously isolated from natural sources. The complete assignment of the ^{13}C and ^{1}H NMR resonances of the β-D-glucopyranosyl esters **1**, **4**, **5** and **6**, given in Table 1 (^{13}C NMR signals), or in Section 4 (^{1}H NMR signals) were in each case substantiated by correlations observed in the two-dimensional HSQC, HMBC, COSY and ROESY spectra of the respective metabolites.

This also appears to be the first report of the isolation from cell suspension cultures of *Solanum* species of a diglucopyranosyl ester, and (in the case of *p*-aminobenzoic acid) the formation of *N*-acetyl and *N*-formyl analogue. We are presently investigating the biotransformation of other substrates by *Solanum* species in order to ascertain whether or not *N*-acetylation or *N*-formylation is a more general biotransformation phenomena in this species.

4. Experimental

4.1. General 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, or at 300.13 MHz (^{1}H) and 75.47 (^{13}C) using a 5 mm QNP probe head installed in a Bruker AC 300 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; ^{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 Fissons VG Platform II instrument. Samples were introduced into the spectrometer using CH_3CN:H_2O (1:1) as solvent. Electron impact mass spectra (EIMS) of **2** and **3** were obtained using a Hewlett Packard (HP) HP5980B mass selective detector (MSD) interfaced to a HP5970 GC fitted with a 25 m × 0.22 mm HP-1 capillary column. The GC oven was temperature programmed from 100° to 250° at 10°/min. The differential scanning (DSC) thermogram of synthetic specimen of **2** was determined using a Shimadzu DT30 differential thermal analyzer.

4.2. Cell suspension culture and biotransformation conditions

Clone sm. of the cell suspension cultures used in these studies were initiated from the callus cultures of *S. mammosum* as previously reported (Syahrani et al., 1997a, 1997b). The calli were cultivated in 300 ml Erlenmeyer flasks containing 50 ml of modified Murashige and Skoog medium (Murashige & Skoog, 1962) 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 (120 rpm) at 25 ± 1° under continuous light (ca. 2000 lux). Biotransformation experiments were typically performed by inoculating cells (10 g fresh weight) into the liquid medium (50 ml) containing **2**, **7** (1000 mg l^{-1}), or **8** (750 mg l^{-1}) and incubated for 7 days. After 7 days the cultures were harvested, filtered, weighed, oven dried at 40° (until their water content was ca. 2%) and powdered.

4.3. Toxicity assessment experiments

Cells (10 g fresh weight) were inoculated into liquid medium (50 ml) containing various concentration of **2**, **7** or **8** (500, 750, 1000, 1250 and 1500 mg l^{-1}) and control cultures (without the addition of **2**, **7** or **8**). After 7 days the cultures were harvested, followed by PCV determination and microscopic observation then filtered, weighed, oven dried at 40° and powdered.

4.4. Biotransformation of 7 and isolation of 1, 2 and 3

Incubation of *p*-aminobenzoic acid (**7**) and work-up of the cell culture as described above, produced powdered biomass (6 g) which was refluxed for 2 h with MeOH. Concentration of the MeOH extract under reduced pressure using a rotatory evaporator, afforded a residue (2.5 g) which was submitted to column chromatography (CC) on Silicagel 40 (Merck, 70–230 mesh) using EtOAc:MeOH:H_2O (77:13:10) as eluent. Purification of the glucopyranosyl fraction by preparative TLC (Silicagel 60 GF 254, Merck; 0.25 mm layer) using EtOAc:MeOH:H_2O (77:13:10) as the developing solvent and UV detection at 254 nm, yielded *p*-aminobenzoic acid 7-*O*-β-D-glucopyranosyl ester (**1**) (310 mg) (R_f 0.37) and material which was shown by one and two-dimensional NMR and GC/MS analyses to be a ca. 3:1 mixture of *N*-acetyl *p*-aminobenzoic acid (**2**) and *N*-formyl *p*-aminobenzoic acid (**3**) (24 mg) (R_f 0.51).

Metabolite **1** decomposed at 190° (DSC analyses); positive ion ESMS (+20 V, Na^+ and NH_4^+ assisted matrix) *m*/*z* (% rel.int.) 300 ($[M+H]^+$, 33), 317 ($[M+NH_4]^+$, 100), 322 ($[M+Na]^+$, 26), 616 ($[2\,M+NH_4]^+$, 26), 621 ($[2\,M+Na]^+$, 14); negative ion ESMS (−110 V, Cl^- assisted matrix) *m*/*z* (% rel.int.) 298 ($[M{-}H]^-$, 100), 334 ($[M+{}^{35}Cl]^-$, 79), 336 ($[M+{}^{37}Cl]^-$, 32), 597 ($[2\,M{-}H]^-$, 61), 633 ($[M+{}^{35}Cl]^-$, 25), 635 ($[M+{}^{37}Cl]^-$, 12). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 3.25 (1H, m, H-3′), 3.32 (1H, m, H-2′), 3.39 (2H, m, H-4′ and H-5′), 3.56 (1H, m, H-$6'_A$), 3.73 (1H, m, H-$6'_B$), 5.59 (1H, d, J = 7.8 Hz, H-1′), 6.13 (2H, s, NH_2), 6.67 (2H, d, J = 8.7 Hz, H-3 and H-5), 7.78 (2H, d, J = 8.7 Hz, H-2 and H-6); ^{13}C NMR: see Table 1.

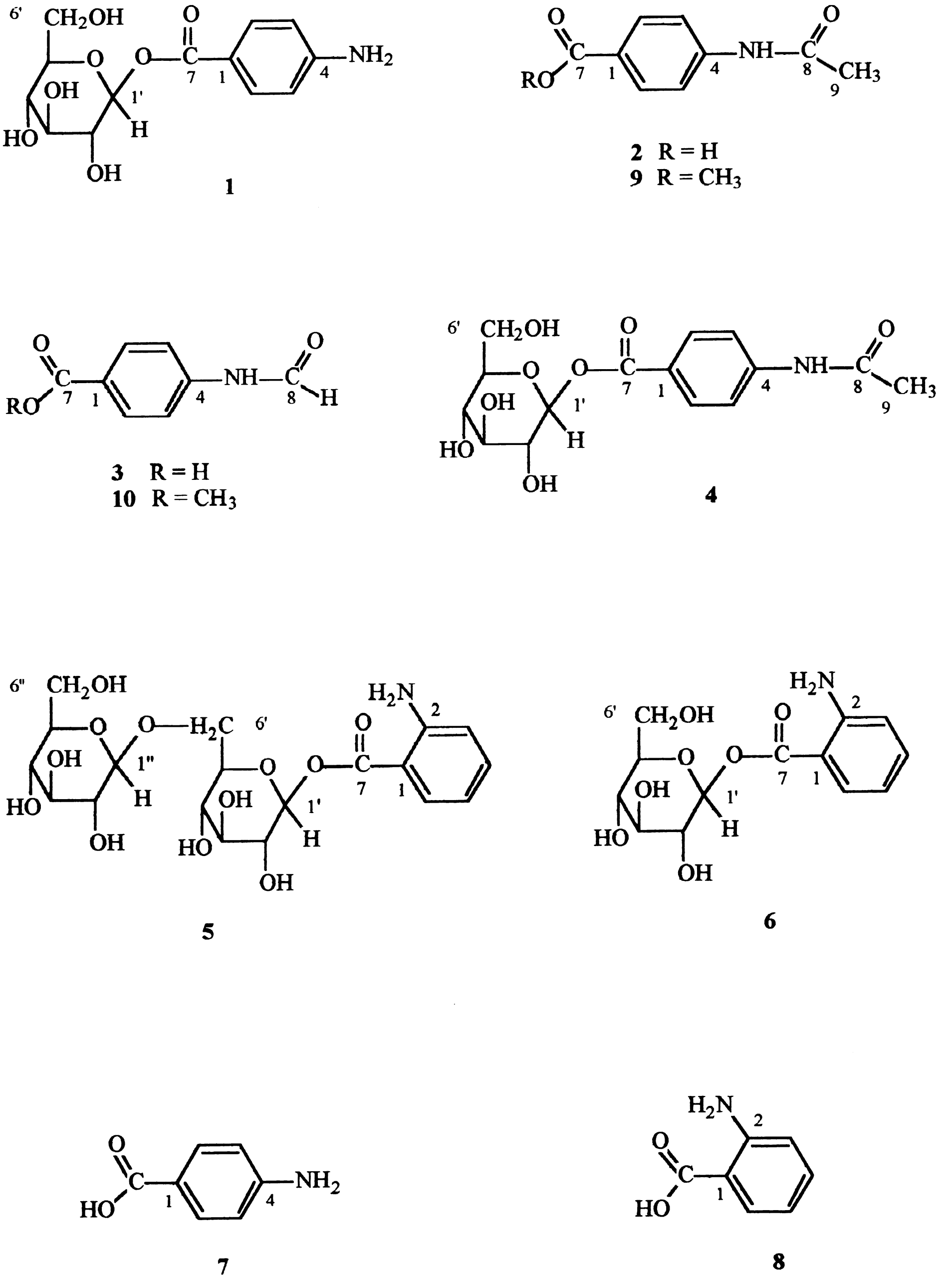
Metabolite **2** had ^{1}H NMR (DMSO-d_6, 400.13 MHz) δ 2.12 (3H, s, $COCH_3$), 7.57 (2H, d, J = 8.4 Hz, H-3 and H-5), 7.88 (2H, d, J = 8.4 Hz, H-2 and H-6), 10.09 (1H, s, NH); ^{13}C NMR (DMSO-d_6, 100.62 MHz) δ 24.0 (C-9), 117.5 (C-3 and C-5), 129.5 (C-2 and C-6, 134.9 (C-1), 139.8 (C-4), 168.2 (C-8), 169.7 (C-7). An authentic specimen of *N*-acetyl *p*-aminobenzoic acid (**2**) was prepared using a published method (Vogel, 1956). The purity of the synthetic product, which exhibited ^{13}C NMR signals identical to observed for the isolated specimen of **2**, was verified by TLC. A sharp endothermic peak in the DSC thermogram of 2 was observed at 147°C.

Metabolite **3** had ^{1}H NMR (DMSO-d_6, 400.13 MHz) δ 7.58 (2H, d, $J = 8.4$ Hz, H-3 and 5), 7.89 (2H, d, $J = 8.4$ Hz, H-2 and H-6), 8.34 (1H, d, $J = 2.0$ Hz, NH–C*H*O), 10.42 (1H, br s, NH); ^{13}C NMR (DMSO-d_6, 100.62 MHz) δ 117.7 (C-3 and C-5), 129.6 (C-2 and C-6), 135.6 (C-1), 138.7 (C-4), 159.2 (C-8), 169.8 (C-7).

GC/MS analyses of a methylated subsample of the mixture of metabolites **2** and **3**, prepared by treatment of a $CHCl_3$ solution of the mixture with an ethereal solution of diazomethane, showed peaks attributable to the presence of *N*-acetyl *p*-aminobenzoic acid methyl ester (**9**), EIMS *m/z* (% rel.int.) 193 (M^+, 29), 151 (57), 120 (100), 92 (23) and *N*-formyl *p*-aminobenzoic acid methyl ester (**10**), EIMS *m/z* (% rel.int.) 179 (M^+, 81), 148 (68), 120 (100), 92 (32).

*4.5. Biotransformation of **2** and isolation of **4***

Incubation of *N*-acetyl *p*-aminobenzoic acid (**2**) and work-up of the cell culture as described above, produced powdered biomass (11.8 g) which was refluxed


1

2 R = H
9 R = CH_3

3 R = H
10 R = CH_3

4

5

6

7

8

Fig. 1. The structure of compounds **1**–**10**.

for 2 h with MeOH. Concentration of the MeOH extract under reduced pressure using a rotatory evaporator afforded a residue (2.01 g) which was submitted to column chromatography Silicagel 40 (Merck, 70–230 mesh) using EtOAc:MeOH:H_2O (77:13:10) as eluent and preparative TLC (Silicagel 60 GF 254, Merck; 0.25 mm layer) using EtOAc:MeOH:H_2O (77:13:10) as the developing solvent and UV detection at 254 nm. Removal of MeOH (used as the elution solvent for the TLC band) yielded *N*-acetyl *p*-aminobenzoic acid 7-*O*-β-D-glucopyranosyl ester (**4**) (117 mg) (R_f 0.25), ^1H NMR (DMSO-d_6, 400.13 MHz) δ 2.16 (1H, s, $COCH_3$), 3.19 (1H, m, H-4′), 3.37 (3H, m, H-2′, H-3′ and H-5′), 3.54 (1H, ddd, $J = 5.7$, 5.7, 11.7 Hz, H-6′), 3.75 (1H, ddd, $J = 1.9$, 5.7, 11.7 Hz, H-6″), 4.63 (1H, t, $J = 5.9$ Hz, 6′-OH), 5.08 (1H, d, $J = 5.4$ Hz, 4′-OH), 5.18 (1H, br d, $J = 3.1$ Hz, 3′-OH), 5.41 (1H, d, $J = 4.7$ Hz, 2′-OH), 5.63 (1H, d, $J = 7.7$ Hz, H-1′), 7.81 (2H, d, $J = 7.8$ Hz, H-3 and H-5), 8.03 (2H, d, $J = 7.8$ Hz, H-2 and H-6), 10.37 (1H, s, NH); ^{13}C NMR: see Table 1.

4.6. Biotransformation of 8 and isolation of 5 and 6

Incubation of *o*-aminobenzoic acid (**8**), and work-up of the cell culture as described above, produced powdered biomass (14.42 g) which was refluxed for 2 h with MeOH. Concentration of the MeOH extract under reduced pressure using a rotatory evaporator, yielded a residue (6.01 g) which was submitted to column chromatography and preparative TLC (Silicagel 60 GF 254, Merck; 0.25 mm layer) using EtOAc:MeOH:H_2O (77:13:10) as the developing solvent and UV detection at 254 nm. *o*-Aminobenzoic acid 7-*O*-β-D-(β-1,6-*O*-D-glucopyranosyl)glucopyranosyl ester (**5**) (32 mg) (R_f 0.08) and *o*-aminobenzoic acid 7-*O*-β-D-glucopyranosyl ester (**6**) (5 mg) (R_f 0.42) were obtained after removal of MeOH (used as the elution solvent for the TLC band).

Metabolite **5** had positive ion ESMS (+100 V, Na^+ assisted matrix) *m*/*z* (% rel.int.) 462 ($[M+H]^+$, 76), 484 ($[M+Na]^+$, 100), 923 ($[2M+H]^+$, 29), 945 ($[2M+Na]^+$, 27); negative ion ESMS (−110 V, Cl^- assisted matrix) *m*/*z* (% rel.int.) 460 ($[M–H]^-$, 100), 496($[M+{}^{35}Cl]^-$, 67), 498 ($[M+{}^{37}Cl]^-$, 30); ^1H NMR (DMSO-d_6, 400.13 MHz) δ 3.04 (1H, t, $J = 8.2$ Hz, H-2″), 3.13 (2H, m, H-4″ and H-5″), 3.38 (1H, m, H-4′), 3.40 (2H, m, H-2′, H-3′), 3.52 (1H, m, H-6″$_A$), 3.54 (1H, m, H-5′), 3.68 (1H, dd, $J = 11.7$, 5.1 Hz, H-6′$_A$), 3.72 (1H, d, $J = 11.9$ Hz, H-6″$_B$), 4.08 (1H, d, $J = 11.7$ Hz, H-6′$_B$), 4.25 (1H, d, $J = 7.7$ Hz, H-1″), 5.61 (1H, d, $J = 7.9$ Hz, H-1′), 6.62 (1H, ddd, $J = 8.2$, 7.0, 1.0 Hz, H-5), 6.74 (2H, br s, NH_2), 6.86 (1H, dd, $J = 8.4$, 1.0 Hz, H-3), 7.36 (1H, ddd, $J = 8.4$, 7.0, 1.6 Hz, H-4), 7.85 (1H, dd, $J = 8.2$, 1.6 Hz, H-6); ^{13}C NMR: see Table 1.

Metabolite **6** had positive ion ESMS (+100 V, Na^+ assisted matrix) *m*/*z* (% rel.int.) 300 ($[M+H]^+$, 3), 322 ($[M+Na]^+$, 35), 599 ($[2M+H]^+$, 14), 621 ($[2M+Na]^+$, 100); negative ion ESMS (−110 V, Cl^- assisted matrix) *m*/*z* (% rel.int.) 298 ($[M–H]^-$, 73), 334 ($[M+{}^{35}Cl]^-$, 100), 336 ($[M+{}^{37}Cl]^-$, 32), 597 ($[2M–H]^-$, 77), 633 ($[2M+{}^{35}Cl]^-$, 60), 635 ($[2M+{}^{37}Cl]^-$, 29); ^1H NMR (DMSO-d_6, 400.13 MHz) δ 3.25 (1H, m, H-4′), 3.30–3.40 (3H, br m, H-2′, H-3′ and H-5′), 3.75 (1H, m, H-6′$_A$), 5.61 (1H, d, $J = 7.9$ Hz, H-1′), 6.62 (1H, ~t, $J = 8.2$ Hz, H-5), 6.73 (2H, br s, NH_2), 6.86 (1H, d, $J = 8.4$ Hz, H-3), 7.35 (1H, ~t, $J = 8.2$ Hz, H-4), 7.86 (1H, d, $J = 8.1$ Hz, H-6), 3.54 (1H, m, H-6′$_B$); ^{13}C NMR: see Table 1. For structures of the compounds used see Fig. 1.

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