

## $\beta$ -GLUCOSYLTRANSFERASE IN CELL CULTURES OF *VERBESINA CARACASANA*

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**Abstract** – (*E*)-3,4-Dimethoxycinnamic acid, inoculated in cell cultures of *Verbesina caracasana*, was converted to the corresponding  $\beta$ -glucopyranoside ester, revealing the presence of an exoglucosyltransferase. An extensive study on other cinnamic acid derivatives showed that the aromatic ring must contain at least one methoxy substituent, but no hydroxy group, for the esterification to be performed. Moreover, the presence of the double bond was shown to have no influence. The glucosylation reaction may involve also the hydroxyl of benzyl alcohols, with the same specificity towards the substitution of the aromatic ring as shown by the cinnamic acids.

Since Yamaha and Cardini<sup>1</sup> found an enzyme from wheat germ that catalyzes the formation of glucosides from phenols and nucleotide activated sugars, it has been demonstrated that a wide variety of higher plants<sup>2</sup> and ferns<sup>3</sup> possess the capacity for glucosylation of administered phenols.

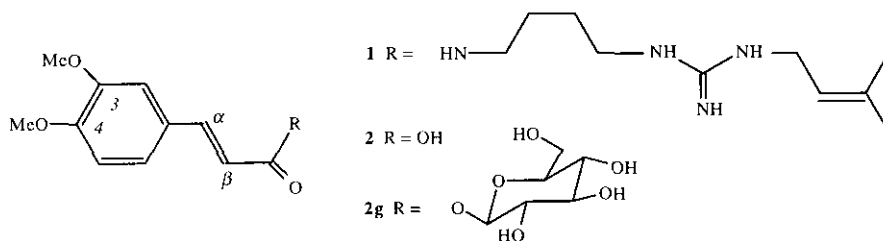
Harborne and Corner<sup>4</sup> reported the formation of glucose esters in leaves of various plants fed with cinnamic acid and its derivatives.

On the other hand, various plant cell cultures were shown to glucosylate exogenous compounds such as simple phenols,<sup>5</sup> coumarins,<sup>6</sup> flavonoids,<sup>7</sup> lignans,<sup>8</sup> steroids,<sup>9</sup> and cardenolides,<sup>10</sup> whereas *p*-hydroxybenzoic acid was transformed in cultured cells of *Mallotus japonicus* besides to the *O*-glucoside to its glucose ester.<sup>11</sup>

A cinnamic glucosyl transferase, which gave (*E*)-cinnamoyl-D-glucose and other sugar derivatives of cinnamate, has been extracted from *Phaseolus vulgaris* and *Medicago sativa*.<sup>12</sup>

This paper deals with a glucosyl transferase activity in whole cells of *Verbesina caracasana* and the results concerning the glucosylation of several exogenous precursors related to cinnamic acids and benzyl alcohols.

Cell suspension cultures of the plant had been established,<sup>13</sup> with the aim to produce two biologically active compounds, namely, caracasamide (G1, **1**)<sup>14</sup> and its cyclobutane dimer caracasandiamide (G2).<sup>15</sup> Since the suspended cells were not able to produce any interesting secondary metabolite, even under stress conditions, biotransformation studies were undertaken using as precursors 3,4-dimethoxycinnamic acid (**2**) and agmatine (1-amino-4-guanidinobutane), as suggested by the biogenetic hypothesis.<sup>16</sup>



In preliminary experiments with whole cell cultures of *Verbesina caracasana* (8 h incubation) neither the target compound G1 was formed, nor the precursors were recovered. By contrast, a new product was isolated from the ethyl acetate extract of whole cells and attributed the structure (*E*)-3,4-dimethoxycinnamic acid  $\beta$ -glucopyranoside ester (**2g**). No similar compound was detected in the spent medium. The structure assignment followed from <sup>1</sup>H- and <sup>13</sup>C-NMR data (Table 1) and a molecular peak at *m/z* 370 in the EIMS spectrum, which indicated the structure of a glucosyl ester. The base peak in the EIMS spectrum (at *m/z* 208) and the successive fragmentation were coincident with those of the acid itself. In the FABMS spectrum a strong  $[\text{M} + \text{Na}]^+$  peak was present and the base peak was found at *m/z* 191, corresponding to the formal loss of OH from the acid. The location of the acyl group was evidenced by the lowfield resonance ( $\delta$  6.56) of the anomeric proton in the <sup>1</sup>H NMR spectrum, while the coupling constant ( $J = 8$  Hz) suggested a  $\beta$  glucosidic linkage. Irradiation of the above signal in a DIFNOE experiment revealed the proximity of H-3' and H-5' protons, confirming the cinnamoyl substituent to be in  $\beta$  orientation. The <sup>1</sup>H- and <sup>13</sup>C-NMR spectra of compound (**2g**) showed the presence (estimated as *ca.* 30% by integration) of the *Z* isomer, characterized by two doublets ( $J = 12$  Hz) at  $\delta$  6.98 and 6.08 in the proton spectrum.

Therefore, we turned our attention to the  $\beta$ -glucosyltransferase responsible for the conversion of **2** to **2g**. A series of experiments were performed to establish the variation of the enzymatic activity at different

Table 1.  $^{13}\text{C}$ - and  $^1\text{H}$ - NMR data of glucosylated cinnamic acid derivatives

Carbon	2g	11g	12g	13g	14g
1	127.59	136.46	112.01	123.12	116.55
2	110.94	106.34	160.43	153.83	160.39
3	150.05	161.40	104.21	112.81	98.85
4	152.24	103.26	132.32	113.60	163.68
5	112.02	161.40	104.21	152.99	106.32
6	123.18	106.34	160.43	117.89	130.98
$\alpha$	146.32	145.95	137.13	141.05	141.55
$\beta$	115.82	118.79	167.70	118.77	115.89
1'	96.18	96.09	95.98	96.04	96.13
2'	74.40	74.12	74.17	74.10	74.38
3'	78.61	78.34	78.41	78.35	78.62
4'	71.10	70.82	70.91	70.81	71.08
5'	79.60	79.40	79.36	79.38	79.59
6'	62.23	61.98	62.09	61.99	62.23
OMe	55.86	55.23	55.76	55.69	55.50
OMe	55.80			55.50	55.31
Proton					
2	7.19 d (2)	6.89 d (2)	-	-	-
3	-	-	6.58 d (8.5)	6.91 d (9)	6.59 d (2)
4	-	6.74 d (2)	7.26 t (8.5)	7.06 dd (9,3)	-
5	6.91 d (8.5)		6.58 d (8.5)		6.63 dd (8.5, 2)
6	7.17 dd (8.5, 2)	6.89 d (2)	-	7.30 d (3)	7.57 d (8)
$\alpha$	7.97 d (16)	7.96 d (16)	8.64 d (16)	8.42 d (16)	8.38 d (16)
$\beta$	6.66 d (16)	6.80 d (16)	7.27 d (16)	6.93 d (16)	6.82 d (16)
1'	6.56 d (8)	6.56 d (7.5)	6.29 d (8)	6.58 d (7.5)	6.60 (7.5)
2'	4.34 t (8.5)	4.34 t (8.5)	4.23 t (8.5)	4.33 br t (8)	4.27 br t (8)
3'	4.38 t (9)	4.37 t (9)	4.28 t (9)	4.35 t (9)	4.36 t (9)
4'	4.40 t (9)	4.39 t (9)	4.26 t (9)	4.38 t (9)	4.39 t (9)
5'	4.14 m (16.5)	4.14 m (16)	4.05 m (16.5)	4.14 m (16.5)	4.13 m (16.5)
6'	4.53 dd (12, 2.5)	4.53 dd (12, 2.5)	4.45 dd (12, 2.5)	4.53 dd (12, 2.5)	4.54 dd (12, 2.5)
	4.43 dd (12, 5)	4.44 dd (12, 4.5)	4.35 dd (12, 5)	4.43 dd (12, 5)	4.44 dd (12, 5)
OMe	3.80.s	3.74 s	3.66 s	3.73 s	3.66 s
OMe	3.74 s			3.65 s	3.65 s

Varian Gemini 300 MHz, in  $\text{C}_5\text{D}_5\text{N}$ , TMS as int. stand. In  $^1\text{H}$  NMR spectrum the signals showed the appropriate integrate intensities. Carbon and proton assignments were supported by HETCOR and INEPTL experiments. Coupling constants ( $\Sigma J$  for multiplets) are given in Hz into parentheses.

ages of the cultures. Cell suspensions, inoculated with (*E*)-3,4-dimethoxycinnamic acid, were grown for different periods (0, 7, 14, 21 and 28 days) ranging from the early lag phase and late stationary phase. The enzymatic activity did not change significantly through the cell growth, little deflection being detected in 28 day old cultures.

The specificity of the  $\beta$ -glucosyltransferase system was then studied by incubation of a series of cinnamic acid derivatives, featuring different substitution patterns in the aromatic ring (Table 2) into 7 day-old cell cultures. Extracts (ethyl acetate) of cells and medium were separated by column chromatography and a series of glucosylated compounds were isolated and identified on the basis of the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectral data (Table 1) and mass fragmentation.

The results, as summarized in Table 2, reveal that substrates with a single methoxy substituent, compounds (4) and (6), are transformed into the corresponding  $\beta$ -glucosyl derivatives in low yield (5-10%). Substrates bearing a hydroxyl substituent either at C-3 or at C-4, compounds (8) and (9), appear to inhibit the glucosylation even when a methoxyl group is present either at C-4 or at C-3, respectively, compounds (10) and (11). Table 2 shows also that the presence of a second methoxy substituent in the aromatic ring (mainly in position 2 or 6) increases significantly the  $\beta$ -glucosylation, compound (12-14) vs (4) and (6).

(*E*)-Cinnamic acid (3) was recovered (30%) from the spent medium together with the (*Z*)-isomer (12%); blank experiments (without cells) confirmed that the *Z-E* isomerization was enzyme-catalyzed and not light induced. Moreover, the extract gave a mixture (*E,Z*) of *p*-hydroxycinnamic acid (8%) by a conversion previously reported in the literature.<sup>12</sup>

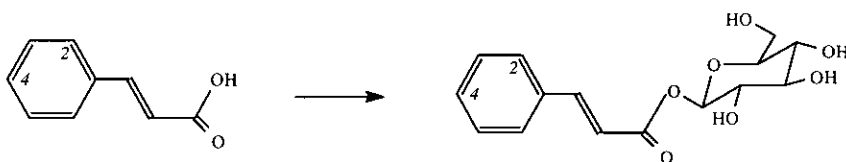
An interesting result was obtained when 2,6-dimethoxyphenylpropionic acid was inoculated in cell cultures of *Verbesina caracasana*. Again the  $\beta$ -glucopyranoside ester was isolated (see Experimental), thus showing that the double bond is not necessary in the precursor for the esterification of glucose.

In most of the experiments with cinnamic acids a second product was isolated assigned the structure  $\beta$ -ethylglucose, by the NMR and MS spectral data (see Experimental). This finding suggested to extend the study on enzyme specificity to benzyl alcohols.

The results shown in Table 3 reveal that the  $\beta$ -glucosyl transferase derived from cell culture of *Verbesina caracasana* is able to catalyse also the etherification reaction of the alcohols (15-18), but the substrates must have in the aromatic ring the same substitution patterns as those required for the esterification of cinnamic acid derivatives.  $^{13}\text{C}$ - and  $^1\text{H}$ -NMR spectral data of the obtained  $\beta$ -glucosides (15g-18g) are summarized in Table 4.

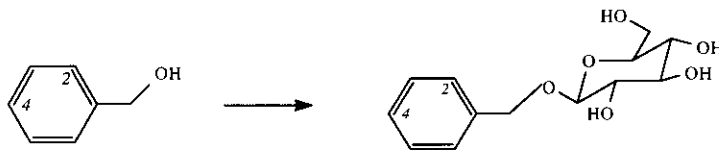
EIMS spectra of products (15g-18g) showed a molecular peak at  $m/z$  330 and the base peak at  $m/z$  151, corresponding to a tropylium ion. In FABMS spectra the base peak is still at  $m/z$  151, while the molecular peak was found at  $m/z$  353 ( $M + \text{Na}^+$ ). Also the  $\beta$ -glucosides (15g-18g) of the alcohols were isolated from the whole cells and were not released into the spent medium.

Table 2. Glucosylation of cinnamic acid derivatives



Substrate	Substitution pattern					Product (yield)
	C-2	C-3	C-4	C-5	C-6	
<b>2</b>	H	OMe	OMe	H	H	<b>2g (28%)</b>
<b>3</b>	H	H	H	H	H	-
<b>4</b>	OMe	H	H	H	H	<b>4g (10%)</b>
<b>5</b>	H	OMe	H	H	H	-
<b>6</b>	H	H	OMe	H	H	<b>6g (7%)</b>
<b>7</b>	H	H	OH	H	H	-
<b>8</b>	H	OH	OH	H	H	-
<b>9</b>	H	OMe	OH	H	H	-
<b>10</b>	H	OH	OMe	H	H	-
<b>11</b>	H	OMe	H	OMe	H	<b>11g (12%)</b>
<b>12</b>	OMe	H	OMe	H	H	<b>12g (17%)</b>
<b>13</b>	OMe	H	H	OMe	H	<b>13g (30%)</b>
<b>14</b>	OMe	H	H	H	OMe	<b>14g (56%)</b>

Table 3. Glucosylation of benzyl alcohol derivatives



Substrate	Substitution pattern					Product (yield)
	C-2	C-3	C-4	C-5	C-6	
<b>15</b>	OMe	OMe	H	H	H	<b>15g (3%)</b>
<b>16</b>	OMe	H	OMe	H	H	<b>16g (8%)</b>
<b>17</b>	OMe	H	H	OMe	H	<b>17g (5%)</b>
<b>18</b>	H	OMe	OMe	H	H	<b>18g (8%)</b>

Table 4.  $^{13}\text{C}$ - and  $^1\text{H}$ -NMR data of glucosylated benzyl alcohol derivatives

Carbon	15	16	17	18
1	112.63.	119.51	128.56	131.42
2	148.82*	158.70	151.24	112.20*
3	148.47*	98.72	114.73	149.45
4	121.88	161.08	113.21	151.08
5	124.20	104.74	154.32	112.76*
6	132.71	130.50	111.56	120.95
7	66.06	65.96	65.98	70.83
1'	104.05	104.09	104.32	103.72
2'	75.33	75.34	75.33	75.27
3'	78.64	78.62	78.66	78.67
4'	71.71	71.71	71.61	71.76
5'	78.64	78.61	78.63	78.62
6'	62.77	62.23	62.71	62.88
OMe	60.76	55.30	55.71	55.93
OMe	55.74		55.59	55.73
Proton				
2	-	-	-	7.27 d (2)
3		6.63 d (2.5)	6.85 d (9)	-
4	6.93 dd (8, 1.5)	-	6.93 dd (9, 3)	-
5	7.08 t (8)	6.59 dd (8, 2.5)	-	6.91 d (8.5)
6	7.45 dd (8, 1.5)	7.20 d (8)	7.73 d (3)	7.09 dd (8.5, 2)
7a	5.32 d (12)	5.26 d (12)	5.30 d (13.5)	5.17 d (12)
7b	5.11 d (12)	5.03 d (12)	5.13 d (13.5)	4.88 d (12)
1'	5.05 d (7.5)	5.05 d (7.5)	5.06 d (7.5)	5.03 d (7.5)
2'	4.15 dd (9, 7.5)	4.14 br dd (9, 7.5)	4.17 br t (8)	4.15 td (7.5, 1.5)
3'	4.30 t (9)	4.30 t (9)	4.32 br t (8.5)	4.31 dd (7.5, 6)
4'	4.31 dd (9, 6)	4.31 br dd (9, 7)	4.28 br t (8.5)	4.28 ddd (8.5, 6, 1.5)
5'	4.00 m (14)	3.99 ddd (7.5, 2.5)	3.96 m (15)	4.01 ddd (8.5, 5.5, 3)
6'a	4.60 dd (12, 2.5)	4.59 dd (12, 2.5)	4.57 dd (12, 1.5)	4.63 dd (12, 3)
6'b	4.44 dd (12, 5)	4.44 dd (12, 2.5)	4.31 dd (12, 5)	4.45 dd (12, 5.5)
OMe	3.85 s	3.71 s	3.68 s	3.74 s
OMe	3.70 s	3.61 s	3.60 s	3.68 s

Varian Gemini 300 MHz, in  $\text{C}_5\text{D}_5\text{N}$ , TMS as int. stand. In  $^1\text{H}$  NMR spectrum the signals showed the appropriate integrate intensities. Carbon and proton assignments were supported by HETCOR and INEPTL experiments. Coupling constants ( $\Sigma J$  for multiplets) are given in Hz into parentheses.

\* In the same column may be interchanged.

While the monosaccharide esters of hydroxycinnamic acids are fairly common in all dicotyledons<sup>17</sup> and phenylethanoid glucosides have been described, sugars ether-linked to benzyl alcohol or ester linked to phenylpropanoate are not known.

The applicability of the glucosyl transferase from *Verbesina caracasana* to various substrates might be important in view of a possible application of the plant cell culture to an efficient production of useful glucosides from natural or synthetic precursors.

It has often be suggested that glucosylation serves as a method for detoxification of harmful phenolic compounds.<sup>18</sup> However, recent studies have demonstrated that glucosylation cannot always be considered as a deactivation process. For example, the glucosides of ferulic acid and dehydrodiconiferylglucosides have been shown to regulate ethylene biosynthesis<sup>19</sup> and cell division,<sup>20</sup> respectively, in plants.

On the other hand, the possibility that bifunctional cinnamic acids could be the cross-bridging molecules between lignin and polysaccharide structures has been proposed.<sup>21</sup> The presence of these acids in both base-labile (ester) and acid-labile (ether) linkages suggested that e. g. ferulic acid ether-linked to lignin formed a cross-bridge to polysaccharide through an ester linkage.<sup>22-24</sup>

The  $\beta$ -glucosyltransferase present in cell cultures of *Verbesina caracasana* for its characteristics seems to belong to the exoglucosyltransferase family, which favours glucosyl incorporation in cell wall material, and may play a role in the process of cell extension.<sup>25</sup>

## EXPERIMENTAL

### *Cell suspension cultures*

Seeds of *Verbesina caracasana*, collected in Valencia (Venezuela), were washed and aseptically transferred to sterile Petri dishes containing 10 ml of MS 62 basal salt medium enriched with sucrose (3%) and solidified with 0.9% (w/v) agar. Explants of roots, stem and leaves were inoculated in the same solid substrate enriched with 2,4-D (2,4-dichlorophenoxyacetic acid, 0.1 ppm), NAA (naphthalenacetic acid, 1 ppm) and kinetin (6-furfurylaminopurine, 0.5 ppm). Induction of callus occurred after 3-5 weeks cultivation in the dark at 25° C. The pH of the basal medium was adjusted for both callus and cell suspension to 5.8 before autoclaving. Suspension cultures were initiated after 3-4 subcultures by transferring callus (1.5 g fresh weight) in 250 mL Erlenmeyer flasks containing 65 mL of a liquid medium (65 mL) containing MS 62 (4.4 g/L), sucrose (3%), 2-4-D (0.1 ppm), NAA (1 ppm) and kinetin. Subculturing was performed every 3 weeks so as to maintain the parent culture.

### *Standard biotransformation procedure and isolation of the products*

The precursor (50 mg), dissolved in EtOH/H<sub>2</sub>O, 1:1 (5 mL), was inoculated in sterile conditions in daily portions (0.5 mg for flask) into 10 flasks containing 7 d-old suspension cultures of *Verbesina caracasana*. The inoculated 23 d-old cell cultures were filtered and both medium and cells were extracted with EtOAc

and MeOH, respectively. The residue of the extracts were purified by column chromatography on silica gel with  $\text{CHCl}_3$ -MeOH, 95:5.

#### *Biotransformation of cinnamic acids*

The glucosylated dimethoxycinnamic acids derivatives (**2g**) and (**11g-14g**) were amorphous solids (*E/Z* mixtures, *ca.* 4:1).  $^1\text{H}$ - and  $^{13}\text{C}$  NMR spectra are reported in Table 1. MS spectra (molecular peak and fragmentation) were all comparable with those of *3,4-dimethoxycinnamic  $\beta$ -glucopyranoside* (**2g**). EIMS *m/z* (rel. int.): 370 (4)  $[\text{M}]^+$ , 352 (2)  $[\text{M} - \text{H}_2\text{O}]^+$ , 208 (100), 193 (27), 191 (34), 163 (6), 133 (11), 91 (14), 73 (41); FABMS *m/z* (rel. int.): 393 (14)  $[\text{M} + \text{Na}]^+$ , 370 (6)  $[\text{M}]^+$ , 209 (39), 207 (31), 197 (8), 191 (100), 181 (15), 165 (22), 163 (18), 133 (14), 91 (100).

#### *2-Dimethoxycinnamic $\beta$ -glucopyranoside* (**4g**):

amorphous solid (*E/Z* mixture, *ca.* 3:1);  $^1\text{H}$  NMR ( $\text{C}_5\text{D}_5\text{N}$ ):  $\delta$  8.40 (1H, d,  $J = 16$  Hz, H- $\alpha$ ), 7.60 (1H, dd,  $J = 8$  and 2 Hz, H-6), 7.36 (1H, td,  $J = 8$  and 2 Hz, H-4), 6.99 (1H, br t,  $J = 8$  Hz, H-5), 6.90 (1H, br d,  $J = 8.5$  Hz, H-3), 6.88 (1H, d,  $J = 16$  Hz), 6.58 (1H, d,  $J = 8$  Hz, H-1'), 4.31 (1H, dd,  $J = 12$  and 2.5 Hz, H-6'a), 4.22 (1H, dd,  $J = 12$  and 5 Hz, H-6'b), 4.17 (1H, t,  $J = 9$  Hz), 4.14 (1H, t,  $J = 9$  Hz, H-3'), 4.04 (1H, dd,  $J = 9$  and 8 Hz, H-2'), 3.89 (1H, ddd,  $J = 9, 5$  and 2.5 Hz, H-5'), 3.51 (3H, s, OMe);  $^{13}\text{C}$  NMR:  $\delta$  166.53 (s, C=O), 158.76 (s, C-2), 141.43 (d, C- $\alpha$ ), 131.72 (d, C-4), 129.34 (d, C-6), 124.22 (s, C-1), 121.13 (d, C-5), 120.22 (d, C- $\beta$ ), 96.27 (d, C-1'), 79.58 (d, C-5'), 78.58 (d, C-3'), 74.34 (d, C-2'), 71.05 (d, C-4'), 62.24 (t, C-6'), 55.46 (q, OMe); EIMS *m/z* (rel. int.): 340 (3)  $[\text{M}]^+$ , 322 (5)  $[\text{M} - \text{H}_2\text{O}]^+$ , 178 (87), 161 (90), 147 (100), 131 (38), 91 (90), 73 (71); FABMS *m/z* (rel. int.): 363 (12)  $[\text{MNa}]^+$ , 341 (4)  $[\text{MH}]^+$ , 179 (28), 161 (100).

#### *4-Dimethoxycinnamic $\beta$ -glucopyranoside* (**6g**):

amorphous solid (*E/Z* mixture, *ca.* 3:1);  $^1\text{H}$  NMR ( $\text{C}_5\text{D}_5\text{N}$ ):  $\delta$  7.97 (1H, d,  $J = 16$  Hz, H- $\alpha$ ), 7.54 (2H, d,  $J = 8.8$  Hz, H-2, H-6), 6.94 (2H, d,  $J = 8.8$  Hz, H-3, H-5), 6.64 (1H, d,  $J = 16$  Hz, H- $\beta$ ), 6.58 (1H, d,  $J = 7.5$  Hz, H-1'), 4.54 (1H, dd,  $J = 12$  and 2.5 Hz, H-6'a), 4.44 (1H, dd,  $J = 12$  and 5 Hz, H-6'b), 4.43-4.33 (3H, m, H-4', H-3', H-2'), 4.14 (1H, ddd,  $J = 8.5, 5$  and 2.5 Hz, H-5'), 3.69 (3H, s, OMe);  $^{13}\text{C}$  NMR:  $\delta$  166.59 (s, C=O), 162.06 (s, C-4), 145.80 (d, C- $\alpha$ ), 130.35 (d, C-2, C-6), 127.29 (s, C-1), 115.75 (d, C- $\beta$ ), 114.84 (d, C-3, C-6), 96.14 (d, C-1'), 79.52 (d, C-5'), 78.55 (d, C-3'), 74.33 (d, C-2'), 71.06 (d, C-4'), 62.21 (t, C-6'), 55.33 (q, OMe); EIMS *m/z* (rel. int.): 340 (7)  $[\text{M}]^+$ , 322 (2)  $[\text{M} - \text{H}_2\text{O}]^+$ , 178 (100), 161 (88), 91 (14), 73 (43); FABMS *m/z* (rel. int.): 363 (17)  $[\text{MNa}]^+$ , 341 (5), 179 (32), 161 (100).

#### *Ethyl $\beta$ -glucopyranoside*:

vitreous solid;  $\alpha_D -12^\circ$  (c 0.1, MeOH);  $^1\text{H}$  NMR ( $\text{C}_5\text{D}_5\text{N}$ ): 4.82 (1H, d,  $J = 7.5$  Hz, H-1'), 4.55 (1H, dd,  $J = 12$  and 2.2 Hz, H-6'a), 4.39 (1H, dd,  $J = 12$  and 5 Hz, H-6'b), 4.26 (1H, br t,  $J = 9$  Hz, H-4'), 4.25 (1H, t,  $J = 9$  Hz), 4.08 (1H, dq,  $J = 10$  and 7 Hz,  $\text{OCH}_a\text{CH}_b$ ), 4.03 (1H, br dd,  $J = 9$  and 7.5 Hz, H-2'), 3.93 (1H, ddd,  $J = 9, 5$  and 2.2 Hz, H-5'), 3.67 (1H, dq,  $J = 10$  and 7 Hz,  $\text{OCH}_a\text{CH}_b$ ), 1.17 (3H, t,  $J = 7$  Hz,



Me);  $^{13}\text{C}$  NMR:  $\delta$  104.43 (d, C-1'), 78.56 (d, C-3'), 78.47 (d, C-5'), 75.18 (d, C-2'), 71.64 (d, C-4'), 64.92 (t, OCH<sub>2</sub>), 62.77 (C-6'), 15.51 (q, Me); EIMS  $m/z$  (rel. int.): 208 (22) [M]<sup>+</sup>, 163 (100) [M - OEt]<sup>+</sup>, 145 (61), 131 (50), 127 (22); FABMS  $m/z$ : 231 [MNa]<sup>+</sup>.

#### *Biotransformation of benzyl alcohols*

The glucosilated benzyl alcohols were amorphous solids;  $\alpha_D$ : **15g**, -32° (c 0.6, MeOH); **16g**, -33° (c 0.8, MeOH); **17g**, -28.5° (c 0.7, MeOH); **18g**, -26° (c 0.6, MeOH);  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR in Table 3. MS spectra were all comparable with those of **18g**: EIMS  $m/z$  (rel. int.): 330 (22) [M]<sup>+</sup>, 196 (5), 180 (5), 168 (7), 151 (100), 137 (23), 121 (46), 91 (22), 73 (23); FABMS  $m/z$  (rel. int.): 353 (21) [MNa]<sup>+</sup>, 330 (12) [M]<sup>+</sup>, 185 (6), 167 (10), 165 (6), 151 (100), 121 (53), 91 (100), 73 (38).

#### *Biotransformation of 2,6-dimethoxyphenylpropionic acid.*

The precursor (48 mg) was inoculated in 16 flasks together with fresh cells (22-d, 1 g) growing in the above reported conditions in daily portions (0.5 mg). After 23 d the material was filtered and medium and cells were extracted with EtOAc and MeOH, respectively. Column chromatography (CHCl<sub>3</sub>/MeOH, 95:5) of the EtOAc residue gave *2,6-dimethoxyphenylpropionic  $\beta$ -glucopyranoside* (17 mg, 20%): amorphous solid;  $\alpha_D$  +7° (c 0.17, MeOH);  $^1\text{H}$  NMR (C<sub>5</sub>D<sub>5</sub>N):  $\delta$  7.20 (1H, t, J = 8 Hz, H-4), 6.59 (2H, d, J = 8 Hz, H-3, H-4), 6.44 (1H, d, J = 8 Hz, H-1'), 4.51 (1H, dd, J = 12 and 2.5 Hz, H-6'a), 4.42 (1H, dd, J = 12 and 5 Hz, H-6'b), 4.37 (1H, t, J = 9 Hz, H-4'), 4.33 (1H, t, J = 9 Hz, H-3'), 4.18 (1H, br t, J = 8.5 Hz, H-2'), 4.08 (1H, ddd, J = 9, 4.5 and 2.5 Hz, H-5'), 3.35 (2H, br t, J = 8 Hz, H<sub>2</sub>-7), 2.81 (2H, br t, J = 8 Hz, H<sub>2</sub>-8);  $^{13}\text{C}$  NMR:  $\delta$  172.70 (s, C=O), 158.69 (s, C-2, C-6), 127.98 (d, C-4), 116.72 (s, C-1), 104.30 (d, C-3, C-5), 96.09 (d, C-1'), 79.49 (d, C-3'), 78.52 (d, C-5'), 74.18 (d, C-2'), 71.07 (d, C-4'), 55.70 (q, OMe), 33.99 (t, C-9), 18.97 (t, C-8); EIMS  $m/z$  (rel. int.): 372 (5) [M]<sup>+</sup>, 354 (2) [M - H<sub>2</sub>O]<sup>+</sup>, 210 (43), 164 (17), 151 (100), 121 (10), 91 (40), 73 (22); FABMS  $m/z$  (rel. int.): 395 (7) [MNa]<sup>+</sup>, 373 (3) [MH]<sup>+</sup>, 355 (10) [MH - H<sub>2</sub>O]<sup>+</sup>, 345 (13) [MH - CO]<sup>+</sup>, 211 (29), 193 (27), 165 (25), 151 (100), 121 (17), 91 (62), 73 (35).

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#### REFERENCES

1. T. Yamaha and C. E. Cardini, *Arch. Biochem. Biophys.*, 1960, **86**, 127.
2. J. B. Pridham, *Phytochemistry*, 1964, **3**, 493.
3. A. D. M. Glass and B. A. Bohm, *Phytochemistry*, 1970, **9**, 2197.
4. J. B. Harborne and J. J. Corner, *Biochem. J.*, 1961, **8**, 242.
5. H. J. Scholten, M. J. Schans, and I. P. M. Somharst, *Plant Cell Tissue Organ Cult.*, 1991, **26**, 173.

6. M. Tamata, Y. Umetani, M. Ooya, and S. Tanaka, *Phytochemistry*, 1988, **27**, 809.
7. E. Lewinsohn, E. Berman, Y. Mazur, and S. Gressel, *Phytochemistry*, 1986, **25**, 2531.
8. W. Van Uden, H. Oeij, H. J. Woerdenbag, and N. Pras, *Plant Cell Tissue Organ Cult.*, 1993, **34**, 169.
9. M. Tabata, Y. Umetani, and M. Ooya, *Phytochemistry*, 1988, **27**, 809.
10. A. W. Alferman, J. Schuller, and E. Reinhard, *Planta Medica*, 1980, **40**, 218.
11. S. Tanaka, K. Hayawaka, Y. Umetani, and M. Tabata, *Phytochemistry*, 1990, **29**, 1555.
12. R. Edwards, M. Mavandad, and R. A. Dixon, *Phytochemistry*, 1990, **29**, 1867.
13. B. Botta, D. Misiti, A. Vitali, G. Delle Monache, S. Persichilli, M. Botta, F. Corelli, and M. Carmignani, *Gazz. Chim. Ital.* 1997, **127**, 305.
14. G. Delle Monache, B. Botta, F. Delle Monache, R. Espinal, C. De Luca, M. Botta, F. Corelli, and M. Carmignani, *J. Med. Chem.* 1993, **36**, 2956.
15. G. Delle Monache, B. Botta, F. Delle Monache, R. Espinal, S.C. De Bonnevaux, C. De Luca, M. Botta, F. Corelli, D. Dei, E. Gacs-Baitz, and M. Carmignani, *Biomed. Chem. Lett.* 1996, **6**, 537.
16. C. R. Bind and T. A. Smith, *Phytochemistry*, 1981, **20**, 2345.
17. P. Molgaard and H. Ravn, *Phytochemistry*, 1988, **27**, 2411.
18. J. B. Pridham, 'Phenolics in Plants in Health and Disease', ed. by J. B. Pridham, 1960, Pergamon Press, Oxford, p. 9.
19. C-Y. Shih, E. B. Dumbroff, and J. E. Thompson, *Plant Physiol.*, 1989, **89**, 1053.
20. A. N. Binns, R. H. Chen, H. N. Wood, and D. G. Lynn, *Proc. Natl. Acad. Sci. U.S.A.*, 1987, **84**, 980.
21. R. E. Brice and I. M. Morrison, *Carbohydr. Res.*, 1982, **101**, 93.
22. A. Scalbert, B. Monties, J. Y. Lallemand, E. Guittet, and C. Rolando, *Phytochemistry*, 1985, **24**, 1359
23. A. Scalbert, B. Monties, E. Guittet, and J. Y. Lallemand, *Holzforschung*, 1986, **40**, 119.
24. K. Iiyama, T. B. Tuyet Lam, and B. A. Stone, *Phytochemistry*, 1990, **29**, 733.
25. A. Darwill, M. McNeil, P. Albersheim, and D. Delmer, In "The Biochemistry of Plants", ed. by P. K. Stumpf and E. E. Conn, Academic press, New York, 1980, Vol. 1, p. 92.

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