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GLYCOSYLATION OF PHENOLIC COMPOUNDS BY ROOT CULTURE OF *PANAX GINSENG**

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Key Word Index—*Panax ginseng*; Araliaceae; ginseng; root culture; biotransformation; glycosylation; glucoside; primeveroside; gentiobioside; 3,5-dimethoxyphenol; methyl salicylate; *p*-hydroxyacetophenone.

Abstract—A root culture of *Panax ginseng* was able to convert 3,5-dimethoxyphenol (taxicatigenin) into its glucoside (taxicatin), primeveroside and gentiobioside, methyl salicylate into its glucoside and gentiobioside, *p*-hydroxyacetophenone into its glucoside (picein), and coniferyl alcohol into dihydroconiferin [3-(3-methoxy-4-*O*-β-D-glucopyranosylphenyl)propan-1-ol]. The conversion ratio and the excretion ratio of the conversion products from methyl salicylate were higher than those from the more polar *p*-hydroxyacetophenone, 3,5-dimethoxyphenol and coniferyl alcohol. Among the conversion products, in particular, the excretion ratio (ER) of a glucoside into the medium decreased in proportion to the quotient of substrate's M_r divided by its RR_t on reversed phase HPLC ($-\mathrm{ER} \propto M_r/RR_t$).

INTRODUCTION

Biological transformation by cell suspension cultures is an important tool in the structural modification of compounds possessing useful therapeutic activities. A great number of biotransformation studies using plant cultured cells have been carried out [1–29]. Plant cultured cells have an ability to convert phenolic compounds into their glucosides [22, 23, 26–29] or gentiobiosides [24, 25]. It has been reported that plant cultured cells have a higher glucosylation activity for compounds having a small M_r and a small number of substituents such as hydroxy groups [22]. To obtain compounds which are pharmacologically more active and which are difficult to synthesize chemically, we have investigated the biotransformation capacity to many natural and synthetic organic compounds of plant cell cultures [4–9, 12, 13].

In our previous papers [30, 31], we investigated the biotransformation of aromatic carboxylic acids in a root culture of *Panax ginseng* C. A. Meyer and reported that the conversion ratio, the kinds and the proportion of binding sugars, and the excretion ratio of conversion products relate to the properties of administered substrates, in particular, the polarity. The root culture of *P. ginseng* also has glycosylation activity towards the phenolic hydroxy group in addition to the formation of sugar esters. The present paper reports on the biotransformation of the phenolic compounds 3,5-dimethoxyphenol (taxicatigenin), methyl salicylate, *p*-hydroxyacetophenone and coniferyl alcohol by the root culture of *P. ginseng*, and the excretion of the conversion products.

RESULTS

After administration of 3,5-dimethoxyphenol (**1**) to the root culture of *Panax ginseng*, compounds **2**–**4** were isolated by a combination of silica gel column chromatography and HPLC (Fig. 1). The FABMS spectrum of **2** showed a peak at *m/z* 317 $[M+Na]^+$. The ^1H and ^{13}C NMR spectra of **2** showed it to be a β-D-glucopyranoside of **1** (Experimental and Table 1), and the mp of **2** was in agreement with that of taxicatin [32]. The FABMS spectra of **3** and **4** showed peaks at *m/z* 449 and 479 $[MH]^+$, respectively, indicating additional pentose and hexose units, respectively, to be attached to **2**. In the ^1H and ^{13}C NMR spectra (Experimental and Table 1), the chemical shifts and the coupling constants of the sugar moiety of **3** were in good agreement with those of primeverose and those of **4** with gentiobiose [30, 33, 34]. The sugars in **3** and **4** appeared to be attached to **1** at its phenolic hydroxy group with β configuration, as judged by the signals assigned to the anomeric protons and carbons of the inner glucose moieties at δ4.94 and 102.5, and 4.97 and 102.5, respectively. Thus **2**–**4** are the β-D-glucopyranoside, β-D-primeveroside and β-D-gentiobioside, respectively, of 3,5-dimethoxyphenol.

On administration of methyl salicylate (**5**), compounds **6** and **7** were isolated (Fig. 1). The FABMS spectra of **6** and **7** showed peaks at *m/z* 315 and 477 $[MH]^+$ indicating one and two molecules of hexose, respectively, to be attached to **5**. The ^1H and ^{13}C NMR spectra of the sugar moieties in **6** and **7** were in good agreement with those of **2** and **4**, respectively. Therefore **6** is the β-D-glucoside of **2** and **7**, β-D-gentiobioside.

Compound **9** was isolated as colourless needles from a root culture previously administered *p*-hydroxyacetophenone (**8**) (Fig. 2). The FABMS spectrum of **9** showed a peak at *m/z* 299 $[MH]^+$. In the ^{13}C NMR spectrum of **9** (Table 1), the presence of signals due to the β-glucopyranosyl unit in addition to those due to **8** showed the

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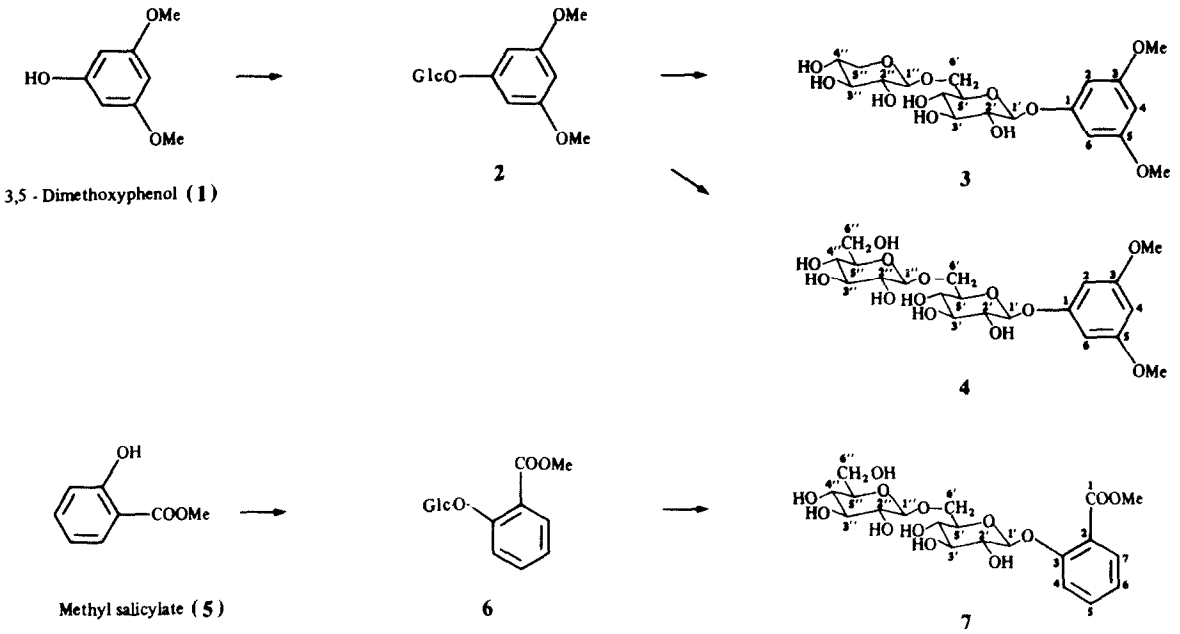

Fig. 1. Biotransformation of 3,5-dimethoxyphenol (**1**) and methyl salicylate (**5**).

Table 1. ^{13}C NMR data for compounds **2–4**, **6**, **7**, **9**, **11** (75 MHz, CD_3OD)

C	2	3	4	6	7	9	11
1	161.3	161.1	161.1	168.9	168.8	26.8	62.5
2	96.8	96.9	96.9	122.7	122.6	200.1	33.0
3	163.2	163.2	163.2	159.0	158.9	133.0	35.9
4	96.0	95.8	95.7	119.3	119.5	132.0	138.8
5	163.2	163.2	163.2	135.4	135.7	117.6	122.2
6	96.8	96.9	96.9	124.0	124.0	163.4	118.6
7				132.3	132.3	117.6	148.8
8						132.0	151.0
9							114.4
1′	102.7	102.5	102.5	104.4	104.1	101.9	103.4
2′	75.2	75.1	75.4	75.3	75.2	75.1	75.3
3′	78.3	78.2	78.1	77.8	77.7	78.3	78.1
4′	71.8	71.4	71.8	71.5	71.6	71.6	71.7
5′	78.6	78.0	77.4	78.8	78.0	78.6	78.5
6′	62.9	70.6	70.5	62.9	70.2	62.8	62.8
1″		105.9	105.2		105.1		
2″		75.2	75.1		75.4		
3″		77.5	78.3		78.3		
4″		71.9	71.9		71.9		
5″		67.2	78.3		78.3		
6″			63.0		63.0		
OMe	56.1	56.1	56.1				57.0
Me				53.1	53.1		

structure of **9** to be the β-D-glucopyranoside of **8**. The mp of **9** was in agreement with that of picein [35].

Compound **11** was isolated as the conversion product from coniferyl alcohol (**10**) (Fig. 2). The FABMS spectrum showed a peak at m/z 367 $[M+Na]^+$. In the ^{13}C NMR of **11** (Table 1), the signals assigned to a β-D-glucopyranosyl unit attached to a phenolic hydroxy group were observed. However the signals of the olefinic carbons in **10** were replaced by two methylene carbons (δ33.0 and 35.9), indicating the olefinic double bond had been reduced. In the ^{1}H NMR, the presence of two methylene protons (δ1.91, 2H, *tt*, $J=7.5$, 6.5 Hz and 2.73, 2H, *t*, $J=7.5$ Hz) confirmed the reduction of the olefinic double bond of coniferyl alcohol. Therefore **11** may be 3-(3-methoxy-4-*O*-β-D-glucopyranosylphenyl) propan-1-ol (dihydroconiferin).

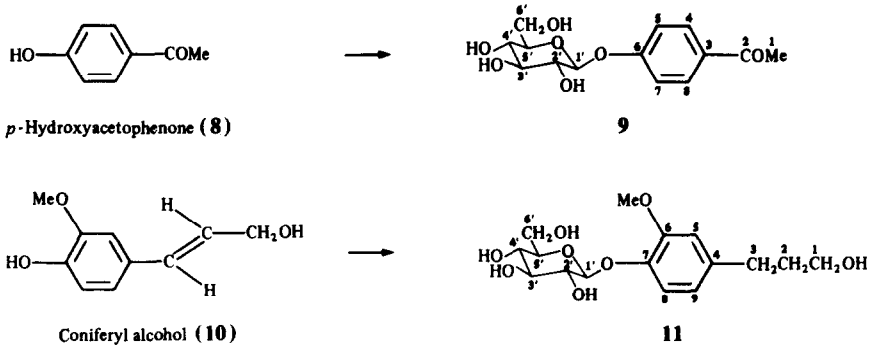

Fig. 2. Biotransformation of p-hydroxyacetophenone (**8**) and coniferyl alcohol (**10**).

DISCUSSION

In the glycosylation of phenolic compounds by the root culture of *P. ginseng*, the conversion ratios obtained for **1**, **5**, **8**, (4-hydroxyphenyl) acetic acid (PHPAA) and ethyl 2-(4-hydroxyphenyl)propionate (PHPA-E) [31] were 22, 54, 14, 13 and 22%, respectively, for a three day incubation period (Table 2). No ratio was obtained for **10**, as the HPLC peak of **11** was too small to provide a reliable quantitative value. The yield of **11** was 0.7%. In the glycosylation of aromatic carboxylic acids by the root culture of *P. ginseng*, the conversion ratios were higher for substrates of lower polarity [31]. To investigate whether a similar relationship between conversion ratio and the polarity of substrate held for the biotransformation of phenolic compounds, the conversion ratios from **1**, **5**, **8**, PHPAA and PHPA-E were compared with their RR_t values on reversed phase HPLC. The results showed that, as in the case of aromatic carboxylic acids, the conversion ratio increased as the polarity (R_t) of the test substrate decreased. It has been reported that for glucosylation of phenolic compounds, the conversion ratio tends to decrease as the number of substituted hydroxy groups is increased, i.e. as the polarity is increased [22]. However, compared with the conversion ratio for an aromatic carboxylic acid having a similar R_t, that for the phenolic compound was under a half. The conversion ratio for **5** after seven days incubation was 63% which is higher than that after three days incubation (Table 2). This result indicated that the root culture of *P. ginseng* biotransforms phenolic compounds more slowly than aromatic carboxylic acids.

The sugars binding to the administered substrates were glucose, primeverose (β-xylose attached to the C-6 position of glucose) and/or gentiobiose [β-(1→6) linked

Table 2. Conversion ratio from each substrate and excretion ratio of the conversion products after three days incubation

Substrates	Products	Conversion ratio (%) R	M	T	Excretion ratio (M/T, %)
1	2	14	1	15	7
	3	5	—	5	0
	4	2	—	2	0
	total	21	1	22	5
5	6	20	27	47	57
		(23)*	(25)	(48)	(52)
	7	7	—	7	0
		(16)	(—)	(16)	(0)
	total	27	27	54	50
		(39)	(25)	(64)	(39)
8	9	13	+	14	+
PHPAA†	12	13	—	13	0
PHPA-E†	13	18	4	22	18

R, in the root; M, in the medium; T, R + M; +, trace amount; **1**,3,5-dimethoxyphenol; **2**, glucoside of **1**; **3**, primeveroside of **1**; **4**, gentiobioside of **1**; **5**, methyl salicylate; **6**, glucoside of **5**; **7**, gentiobioside of **5**; **8**, *p*-hydroxyacetophenone; **9**, glucoside of **8**; PHPAA, (4-hydroxyphenyl) acetic acid; **12**, glucoside of PHPAA; PHPA-E, ethyl 2- (4-hydroxyphenyl) propionate; **13**, glucoside of PHPA-E.

*Values in parentheses indicate the conversion or excretion ratio after seven days incubation.

†see ref. [31].

glucobiose]. In the formation of the sugar esters of aromatic carboxylic acid by the root culture of *P. ginseng*, the sugars found were glucose, primeverose and/or sophorose [β-(1→2) linked glucobiose]. Although glucose and primeverose attached to both carboxylic and phenolic hydroxy groups, in the formation of glucobiosides, gentiobiose selectively attached to the phenolic hydroxy group and sophorose to the carboxylic group. The plant [36–39], callus or root culture [40–42] of *P. ginseng* produce ginsenosides, pharmacologically active saponins which contain sophorose attached to an alcoholic hydroxy group. Therefore the root culture of *P. ginseng* seems to bind mainly sophorose, as a disaccharide, to an alcoholic hydroxy group. With regard to the biotransformation of aromatic carboxylic acids, our previous paper reported that the proportion of disaccharide ester was higher following the administration of a substrate having a smaller polarity. On the administration of **8** or **10**, compounds with short R_ts, only glucose was attached to the substrate. On the other hand, primeverose and gentiobiose, in addition to glucose, were attached to **1** which has a larger R_t than either **8** or **10**, and gentiobiose, in addition to glucose, was attached to **5** which has the highest R_t of the substrates used in this study. Thus, the sugars binding to the substrates show a similar tendency to those observed on administration of aromatic carboxylic acid, though, on administration of PHPA-E having a longer R_t than **1**, attachment of disaccharide was not observed [31].

The root culture of *P. ginseng* did not excrete the conversion product (**11**) from **10**, which has the shortest R_t among the substrates used in this experiment, into the medium, and the excretion of converstion products (**2** and **9**) from **1** and **8** was small. On the other hand, upon administration of **5**, although the gentiobioside (**7**) like the primeroside (**3**) and gentiobioside (**4**) of **1** was not excreted into the medium, over 50% of the glucoside (**6**) was excreted. Therefore the excretion ratio from a substrate having a long R_t (i.e. smaller polarity) is higher as in the case of the excretion ratio of the conversion products from an aromatic carboxylic acid [31]. In particular, the excretion ratio (ER) of glucosides into the medium decreased in proportion to the quotient of the M_r divided by the RR_t of the substrate on reversed phase HPLC ($-ER \propto M_r/RR_t$).

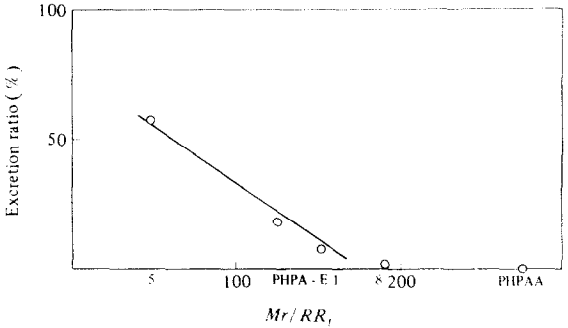

Fig. 3. Relation between excretion ratio of glucosides and M_r and RR_t of substrates. RR_t (benzoic acid = 1, HPLC: column, Senshu-Pak ODS-4301-N; eluent, MeOH–H_2O, 1:1); **1** 3,5-dimethoxyphenol, **5** methyl salicylate; **8**, *p*-hydroxyacetophenone; PHPA-E, 2-(4-hydroxyphenyl) propionic acid; PHPAA, (4-hydroxyphenyl) acetic acid.

EXPERIMENTAL

Mps: uncorr. NMR: CD_3OD.

Administration method. The root culture of *Panax ginseng* was cultured on Murashige and Skoog's agar medium containing IBA (5 ppm) and *N*-phenyl-*N'*-(4-pyridyl)urea (0.1 ppm) for three weeks [30] on a rotary shaker (145 rpm) at 25° in the dark. After three weeks, each test substrate (25 mg) was dissolved in 2 ml EtOH and added to a 250 ml suspension culture which was then cultured for a further three days. The substrates used in these experiments were *p*-hydroxyacetophenone, methyl salicylate, purchased from Wako Pure Chemical (Osaka), and 3,5-dimethoxyphenol and coniferyl alcohol purchased from Aldrich Chem.

Isolation of conversion products. The root cultures administered the test substrates were divided into media and roots by filtration through nylon cloth. The roots were homogenized in MeOH, the homogenate filtered, and the filtrate concd, dissolved in H_2O and combined with the medium. The soln was applied to a column of Diaion HP-20 and washed with H_2O followed by elution with MeOH. From the eluates, the conversion products were isolated by a combination of silica gel CC, HPLC and/or crystallization. The solvent used for silica gel CC was $CHCl_3$–MeOH (6:1 for **2–4**, **9** and **11**). HPLC: Senshu-Pak ODS-4301-N (300 × 10 mm) with MeOH–H_2O (3:7 for **2–4**, **7** and **9**; 1:4 for **11**). From these eluents **2**, **3**, **6**, **7** and **11** were crystallized from MeOH, and **9** from H_2O.

Quantitative analysis of conversion products. The media and MeOH extracts of the roots from 250 ml of suspension culture which had been incubated with the test substrates were dissolved in H_2O. The soln was applied to a column of Diaion HP-20 and washed with water followed by elution with MeOH. The amount of each of the conversion product present in the MeOH eluate was determined by HPLC: Senshu-Pak ODS-4301-N column (300 × 10 mm), MeOH–H_2O (3:7), detection by differential refractometer and UV (254 nm) absorption.

3,5-*Dimethoxyphenoxy* β-D-*glucopyranoside* (*taxicatin*, **2**). Colourless needles: mp 170–171°; $[\alpha]_D^{22} -64.3°$ (MeOH; *c* 0.93); IR ν_{max}^{KBr} cm^{-1}: 3380, 1600; ^{1}H NMR (CD_3OD): δ6.40 (2H, *d*, *J* = 2 Hz, H-2 and 6), 6.24 (1H, *t*, *J* = 2 Hz, H-4), 4.94 (1H, *d*, *J* = 8 Hz, H-1'), 3.98 (1H, *dd*, *J* = 12, 2 Hz) and 3.77 (1H, *dd*, *J* = 12, 5.5 Hz, H-6'), 3.83 (6H, *s*, OMe), 3.38–3.59 (4H, *m*, H-2',3',4' and 5'); ^{13}C NMR (CD_3OD): see Table 1; FABMS *m/z*: 317 $[M+Na]^+$.

3,5-*Dimethoxyphenoxy* 6-O-β-D-*xylopyranosyl*-β-D-*glucopyranoside* (**3**). Colourless needles: mp 216–217°; $[\alpha]_D^{22} -60.0°$ (MeOH; *c* 0.60); IR ν_{max}^{KBr} cm^{-1}: 3420, 1605; ^{1}H NMR (CD_3OD): δ6.41 (2H, *d*, *J* = 2 Hz, H-2 and 6), 6.26 (1H, *t*, *J* = 2 Hz, H-4), 4.94 (1H, *d*, *J* = 7.5 Hz, H-1'), 3.75 (1H, *ddd*, *J* = 9.5, 6.5, 2 Hz, H-5'), 4.19 (1H, *dd*, *J* = 11.5, 2 Hz) and 3.89 (1H, *dd*, *J* = 11.5, 6.5 Hz, H-6'), 4.38 (1H, *d*, *J* = 7.5 Hz, H-1''), 3.29 (1H, *dd*, *J* = 9, 7.5 Hz, H-2''), 3.58 (1H, *ddd*, *J* = 10.5, 8, 5 Hz, H-4''), 3.94 (1H, *dd*, *J* = 11.5, 5 Hz) and 3.24 (1H, *dd*, *J* = 11.5, 10.5 Hz, H-5''), 3.85 (6H, *s*, OMe), 3.36–3.56 (4H, *m*, H-2',3',4' and 3''); ^{13}C NMR (CD_3OD): see Table 1; FABMS *m/z*: 449 $[MH]^+$.

3,5-*Dimethoxyphenoxy* 6-O-β-D-*glucopyranosyl*-β-D-*glucopyranoside* (**4**). Amorphous solid: $[\alpha]_D^{23} -36.6°$ (MeOH; *c* 0.29); IR ν_{max}^{KBr} cm^{-1}: 3430, 1615; ^{1}H NMR (CD_3OD): δ6.41 (2H, *d*, *J* = 2 Hz, H-2 and 6), 6.26 (1H, *t*, *J* = 2 Hz, H-4), 4.97 (1H, *d*, *J* = 8 Hz, H-1'), 3.31 (1H, *dd*, *J* = 9, 8 Hz, H-2'), 4.25 (1H, *dd*, *J* = 11.5, 2 Hz) and 3.91 (1H, *dd*, *J* = 11.5, 6 Hz, H-6'), 4.43 (1H, *d*, *J* = 7.6 Hz, H-1''), 3.95 (1H, *dd*, *J* = 12, 2 Hz) and 3.75 (1H, *dd*, *J* = 12, 5.5 Hz, H-6''), 3.84 (6H, *s*, OMe), 3.34–3.59 (7H, *m*, H-3',4',5',2'',3'',4'' and 5''); ^{13}C NMR (CD_3OD): see Table 1; FABMS *m/z*: 479 $[MH]^+$.

Methyl 2-*O*-β-D-*glucopyranosylbenzoate* (**6**). Colourless needles: mp 69–70°; $[\alpha]_D^{25} -47.0°$ (MeOH; *c* 1.05); IR ν_{max}^{KBr} cm^{-1}:

3420, 1720; ^{1}H NMR (CD_3OD): δ7.49 (1H, *dd*, J=8.5, 1 Hz, H-4), 7.22 (1H, *ddd*, J=8.5, 7.5, 1 Hz, H-5), 7.63 (1H, *ddd*, J=8.5, 7.5, 2 Hz, H-6), 7.85 (1H, *dd*, J=7.5, 2 Hz, H-7), 4.98 (1H, *d*, J=7.5 Hz, H-1'), 4.01 (1H, *dd*, J−12, 2 Hz) and 3.81 (1H, *dd*, J=12, 5.5 Hz, H-6'), 3.98 (3H, *s*, CH_3), 3.46–3.65 (4H, *m*, H-2',3',4' and 5'); ^{13}C NMR (CD_3OD): see Table 1; FABMS *m/z*: 337 $[M+Na]^+$.

Methyl 2-O-(6-O-β-D-*glucopyranosyl*-β-D-*glucopyranosyl*)-*benzoate* (**7**). Colourless needles: mp 147–149°; $[\alpha]_D^{24}$ −39.0° (MeOH; *c* 0.78); IR ν_{max}^{KBr} cm^{-1}: 3405, 1710; ^{1}H NMR (CD_3OD): δ7.55 (1H, *dd*, J=8.5, 1 Hz, H-4), 7.21 (1H, *ddd*, J=8.5, 7.5, 1 Hz, H-5), 7.67 (1H, *ddd*, J=8.5, 7.5, 2 Hz, H-6), 7.85 (1H, *dd*, J=7.5, 2 Hz, H-7), 4.99 (1H, *d*, J=8 Hz, H-1'), 3.32 (1H, *dd*, J=9, 8 Hz, H-2'), 3.79 (1H, *ddd*, J=9, 6, 2 Hz, H-5'), 4.28 (1H, *dd*, J=11.5, 2 Hz) and 3.93 (1H, *dd*, J=11.5, 6 Hz, H-6'), 4.49 (1H, *d*, J=7.6 Hz, H-1''), 3.31 (1H, *dd*, J=9, 7.6 Hz, H-2''), 3.95 (1H, *dd*, J=12, 2 Hz) and 3.74 (*dd*, J=12, 5.5 Hz, H-6''), 3.98 (3H, *s*, Me), 3.38–3.62 (5H, *m*, H-3',4',3'',4'' and 5''); ^{13}C NMR (CD_3OD): see Table 1; FABMS *m/z*: 499 $[M+Na]^+$.

4-O-β-D-*Glucopyranosylacetophenone* (*picein*, **9**). Colourless needles: mp 195–196°; $[\alpha]_D^{24}$ −81.5° (MeOH; *c* 0.14); IR ν_{max}^{KBr} cm^{-1}: 3380, 1665, 1605, 1585, 1515; ^{1}H NMR (CD_3OD): δ2.66 (3H, *s*, H-1), 5.13 (1H, *d*, J=8 Hz, H-1'), 4.00 (1H, *dd*, J=12, 2 Hz) and 3.80 (1H, *dd*, J=12, 5 Hz, H-6'), 8.05–8.10 (2H, *m*, H-4 and 8), 7.24–7.29 (2H, *m*, H-5 and 7), 3.46–3.63 (4H, *m*, H-2',3',4' and 5'); ^{13}C NMR (CD_3OD): see Table 1; FABMS *m/z*: 299 $[MH]^+$.

3-(3-*methoxy*-4-O-β-D-*glucopyranosylphenyl*) *Propan*-1-*ol* (**11**). Colourless needles: mp 77–79°; $[\alpha]_D^{23}$ −50.0° (MeOH; *c* 0.04); IR ν_{max}^{KBr} cm^{-1}: 3430; ^{1}H NMR (CD_3OD): δ3.65 (2H, *t*, J=6.5 Hz, H-1), 1.91 (2H, *tt*, J=7.5, 6.5 Hz, H-2), 2.73 (2H, *t*, J=7.5 Hz, H-3), 6.95 (1H, *d*, J=2 Hz), 7.17 (1H, *d*, J=8 Hz) and 6.83 (1H, *dd*, J=8, 2 Hz, H-5, 8 or 9), 4.94 (1H, *d*, J=8 Hz, H-1'), 3.96 (1H, *dd*, J=12, 2 Hz) and 3.78 (1H, *dd*, J=12, 5 Hz, H-6'), 3.94 (3H, *s*, OMe), 3.33–3.58 (4H, *m*, H-2',3', 4' and 5'); ^{13}C NMR (CD_3OD): see Table 1; FABMS *m/z*: 367 $[M+Na]^+$.

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