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BIOTRANSFORMATION OF AROMATIC CARBOXYLIC ACIDS BY ROOT CULTURE OF *PANAX GINSENG**

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Key Word Index—*Panax ginseng*; plant tissue culture; root culture; biotransformation; glycosylation; aromatic carboxylic acid; glucoside; glucosyl ester; sophorosyl ester.

Abstract—Root culture of *Panax ginseng* is able to convert aromatic carboxylic acids into glucose and/or sophorose substituted conjugates. 2-(4-hydroxyphenyl) Propionic acid is converted into its *R* and *S* glucosyl esters and glucosides; its ethyl ester into a glucoside and a small amount of a glucosyl ester; (4-hydroxyphenyl)acetatic acid into its glucoside; tropic acid into its glucose esters and glucosides; and 2-(3-benzoylphenyl)propionic acid and 2-[2-(6-methoxy)-naphthyl]propionic acid, into their glucosyl and sophorosyl esters. These conversion products are all new compounds. The conversion ratio and binding sugars depend on the properties of the administered substrate, in particular, its polarity and toxicity. Unless the root culture is injured by substrate toxicity,the conversion ratio from substrates having smaller polarities are higher and in some cases 100%. Among the conversion products, the excretion ratio of glycosyl esters into the medium decreased in proportion to the quotient of the M_r divided by the RR_f of substrate in a reversed phase HPLC. In the conversion of (*RS*)-2-(4-hydroxyphenyl)propionic acid into its glucoside[(2*R*)-2-(4-O-β-D-glucopyranosylphenyl)propionic acid], the root culture selectivity converted the *R* isomer, although the formation of glucosyl esters was not selectively performed.

INTRODUCTION

Biological transformations by cell suspension cultures serve as important tools in the structural modification of compounds possessing useful therapeutic activity. A great number of biotransformation studies have been carried out with plant cells in culture [1–24]. To obtain compounds pharmacologically more active and difficult to synthesize chemically, we have investigated the ability of plant cell cultures to biotransform many organic natural and synthetic compounds [4–9, 12, 13]. In our previous papers [25–27], on the biotransformation of 2-phenylpropionic acid, we reported that plant cell cultures have the ability to form sugar esters of 2-phenylpropionic acid. Thus suspension cultures of *Dioscoreophyllum cumminsii* and *Nicotiana tabacum* [25] converted 2-phenylpropionic acid into its glucosyl and gentiobiosyl esters, that of *Aconitum japonicum* [25] into ethyl 6-*O*-(2-phenylpropionyl)-β-D-glucopyranoside and that of *Coffea arabica* [26] into its sucrose ester as main product. In particular, root culture of *Panax ginseng* was very efficient at forming glucose esters (and primeverosyl and inositol esters) of 2-phenylpropionic acid. As about a half the amount of the glucose ester were excreted into the medium this made this system a very useful one for glycosylation [27].

The present paper reports on the biotransformation of various aromatic carboxylic acids[(*RS*)-2-(4-hydroxyphenyl)propionic acid and its ethyl ester, (4-hydroxyphenyl)acetic acid, (*RS*)-tropic acid, (*RS*)-2-(3-benzoylphenyl)propionic acid and (*S*)-2-[2-(6-methoxy)-naphthyl]propionic acid] by root culture of *P. ginseng* (Pg-1) [28]. The biotransformation of various substrates, group selectivity on glycosylation of substrates, having two groups(carboxylic group and phenolic or alcoholic hydroxy groups) and the effect of the substrates and products for the excretion of products into the medium were investigated.

*Part 60 in the series 'Studies on Plant Tissue Culture'. For part 59, see ref. [27].

RESULTS

Biotransformation of (RS)-2-(4-*hydroxyphenyl*)*propionic acid* (1)

On administration of (*RS*)-2-(4-hydroxyphenyl)-propionic acid (1) to the root culture of *P. ginseng*, compounds **2–5** were isolated by silica gel column chromatography and HPLC (Fig. 1). The FAB MS spectra of **2** and **3** showed peaks at *m/z* 351 $[M+Na]^+$. In the ^1H and ^{13}C NMR spectra (Experimental and Table 1) the chemical shifts and coupling constants in the sugar moieties of **2** and **3** were in good agreement with those of β-D-glucopyranose [25, 29, 30]. However, the acylation shifts (about $\delta+1.1$) for the anomeric protons of glucose moieties suggested that the 2-(4-hydroxyphenyl)-propionyl groups were attached to the anomeric positions of the glucose residues. The difference of the chemical shift values between **2** and **3** differed slightly. These differences possibly arise from those in the configuration at C-2 of the 2-(4-hydroxyphenyl)propionyl groups in **2** and **3** were determined by CD spectra. The CD spectrum of **2** showed a positive maximum at 232 nm ($\Delta\varepsilon$: +3.15) and that of **3** a negative maximum at 232 nm ($\Delta\varepsilon$: −2.70). The CD spectrum of **2** exhibited the same Cotton effect as

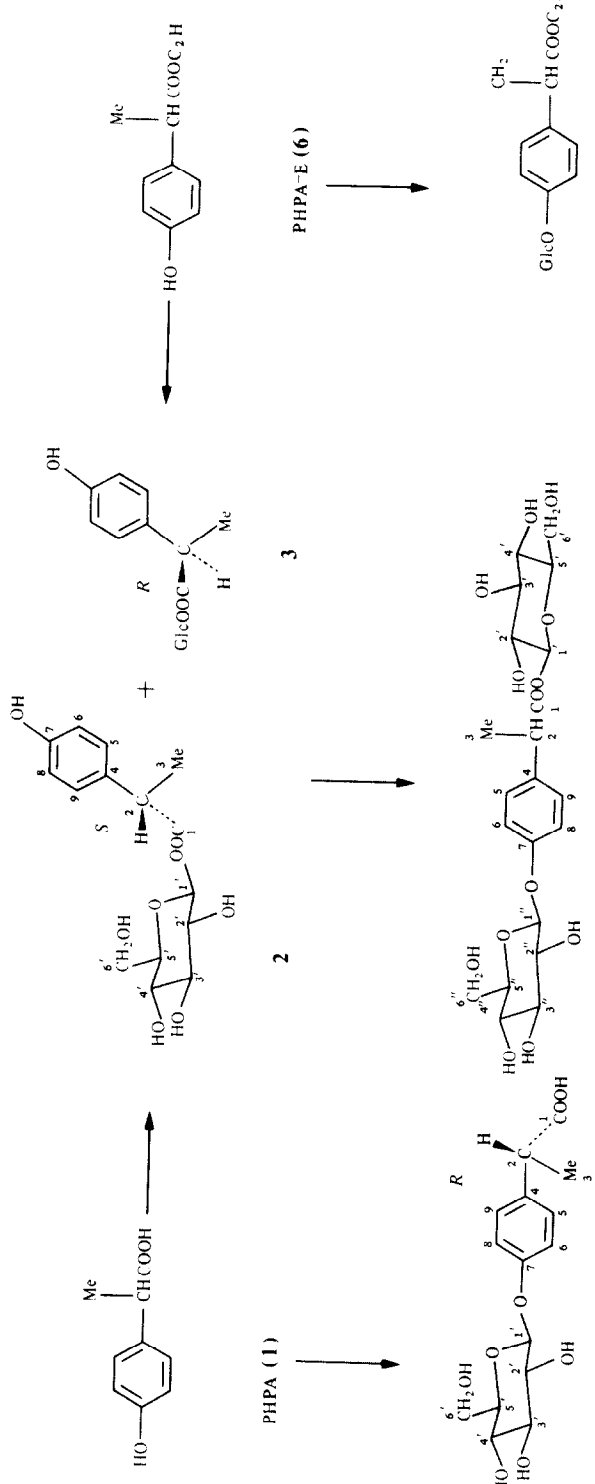

Fig. 1. Possible scheme for the biotransformation of 2-(4-hydroxyphenyl)propionic acid (**1**) and its ethyl ester (**6**) and (4-hydroxyphenyl)acetic acid (**8**) by the root culture of *P. ginseng*.

(2*S*)-2-phenylpropionyl β-D-glucopyranoside produced from (*S*)-2-phenylpropionic acid by biotransformation using the root culture of *p. ginseng* and that of **3**, the reverse, indicating the configuration of **2** to be *S* and that of **3**, *R*. From these results, **2** was assigned the structure of (2*S*)-2-(4-hydroxyphenyl)propionyl β-D-glucopyranoside and **3**, (2*R*)-2-(4-hydroxyphenyl)propionyl β-D-glucopyranoside. The FABMS spectra showed a peak at *m/z* 351 $[M+Na]^+$. The NMR spectra of **4** showed that the composition of **4** was β-D-glyucopyranose and 2-(4-hydroxyphenyl)propionic acid. However, the chemical shifts (δ5.01 and 102.6) of the anomeric proton and carbon indicated that the glucose moiety was attached to the phenolic hydroxy group of the 2-(4-hydroxyphenyl)propionyl group. The CD spectrum of **4** showed a negative maximum at 220 nm ($\Delta\varepsilon$ −1.74) indicating the configuration at C-2 of the 2-(4-hydroxyphenyl)propionic acid moiety of **4** to be *R*. Thus **4** may be (2*R*)-2-(4-*O*-β-D-glucopyranosylphenyl) propionic acid. The FABMS spectrum of **5** showed a peak at *m/z* 513 $[M+Na]^+$, which was larger by 162 mass units than those of **2**–**4**, suggesting the presence of an additional hexose unit in this compounds. In the NMR spectra of **5**, the chemical shifts and the coupling constants of one hexose moiety were in agreement with those of **2** and **3** and those of the other in agreement with those of **4**. Two signals assigned to a methyl function (δ1.55 and 1.51) in the ^{1}H NMR spectrum (Experimental), and the ^{13}C NMR spectrum (Table 1) indicated **5** to be a mixture of the C-2 diastereoisomers of the (*RS*)-2-(4-hydroxyphenyl)propionyl group. By comparison with the ^{1}H NMR spectra of **2** and **3**, the signals at δ1.55 and 1.51 may be assigned to the methyl function of the (2*R*)- and (2*S*)-(4-hydroxyphenyl)propionyl group respectively. Therefore **5** may be a mixture of (2*RS*)-2-(4-*O*-β-D-glucopyranosylphenyl) propionyl β-D-glucopyranoside, with the ratio of 4:1 according to the integrated intensity of the methyl protons.

Biotransformation of ethyl (RS)-2-(4-*hydroxyphenyl*)-*propionate* (**6**)

On administration of the ethyl ester of **1**(**6**), compound **7** in addition to a small amount of a mixture of **2** and **3**, was isolated (Fig. 1). The FABMS spectrum of **7** showed a peak at *m/z* 379 $[M+Na]^+$ which was larger by 28 mass units than **3**. The NMR spectra of **7** exhibited signals (δ4.09 and 4.07, each 0.8 H, *q*, *J* = 7.2 Hz, 4.11 and 4.05, each 0.2 H, *q*, *J* = 7.2 Hz and 1.17, 3H, *t*, *J* = 7.2 Hz in the ^{1}H NMR and δ62.1 and 14.7 in the ^{13}C NMR) showing the presence of ethyl ester, in addition to being in good agreement with those of **4**. In the ^{1}H NMR spectrum, the presence of four signals assigned to methylene protons carrying an oxygen function (δ4.11, 4.09, 4.07 and 4.05) indicated **7** to be a mixture of the diastereoisomers of (*RS*)-ethyl 2-(4-*O*-β-D-glucopyranosylphenyl)propionate, with the ratio of 4:1 according to the integrated intensity of the methylene protons. The CD spectra of **7** showed a negative maximum at 226 nm ($\Delta\varepsilon$ −2.16) indicating the configuration at C-2 of the (*RS*)-2-(4-hydroxyphenyl)propionyl group of **7** was predominantly *R*.

Biotransformation of (4-*hydroxypheynl*)*acetic acid* (**8**)

Compound **9** was isolated as a conversion product from (4-hydroxyphenyl)acetic acid (**8**) (Fig. 1). The FABMS spectrum of **9** showed a peak at *m/z* 337 $[M+Na]^+$, which was smaller by 14 mass units than that of **4**. The NMR spectra of the sugar moiety of **9** was in agreement with that of **3**, and hence **9** may be (4-*O*-β-D-glucopyranosylphenyl)acetic acid.

Biotransformation of (RS)-*tropic acid* (**10**)

Compounds **11**–**15** were isolated as conversion products from (*RS*)-tropic acid (**10**) (Fig. 2). The FABMS spectra of **11**–**15** each showed a peak at *m/z* 351 $[M+Na]^+$, which was larger by 162 mass units than that of tropic acid. The NMR spectra showed that **11**–**15** were composed of β-D-glucopyranose and tropic acid. In the ^{1}H NMR spectra of **11** and **12**, the acylation shifts (*ca* δ +1.1) for the anomeric protons of the glucose moieties suggested that the tropoyl groups were attached to the anomeric positions of the glucose residues. The chemical shifts between **11** and **12** differed slightly. The differences possibly arise from those in the configuration at C-2 of the tropoyl group. The CD spectrum of **11** showed a negative maximum at 218 nm ($\Delta\varepsilon$ −1.91) and that of **12**, a positive maximum at 219 nm ($\Delta\varepsilon$ +3.83), indicating the configuration of **11** to be *S* and that of **12**, *R*. The NMR spectra suggested **13** to be a mixture an α- and a β-anomer of a glucose moiety and the diastereoisomers of (*RS*)-tropic acid. The ratio of the α-and β-anomers was judged to be about 3:2 from the intensity of the respective anomeric protons. In the ^{1}H NMR spectrum of **13**. The H-2 signals of the glucose moiety were shifted downfield (δ4.73, 0.6H, *dd*, *J* = 10, 3.6 Hz and 4.82, 0.4H, *dd*, *J* = 9.5, 8 Hz) as compared with those of **11**, indicating the (*RS*)-tropoyl group to be attached to the C-2 position of a glucose moiety. In the ^{13}C NMR spectra of **14** and **15**, glycosylation shifts (*ca* δ +9, −2 and +7, respectively) of the anomeric carbon of the glucose moiety and the C-2 and 3 of tropic acid, respectively, were observed, indicating the glucose residue to be attached to the C-3 position of tropic acid. The CD spectrum of **14** showed a negative maximum at 218 nm ($\Delta\varepsilon$ −4.12) and that of **15**, a positive maximum at 218 nm ($\Delta\varepsilon$ +4.40), indicating the configuration at C-2 of the 3-hydroxy-2-phenylpropionyl group of **14** to be *S* and that of **15**, *R*. From these result, **11** was assigned of the structure of (2*S*)-3-hydroxy-2-phenylpropionyl β-D-glucopyranoside, **12**, (2*R*)-3-hydroxy-2-phenylpropionyl β-D-glucopyranoside, **13**, (2*RS*)-2-*O*-(3-hydroxy-2-phenylpropionyl)-D-glucose, **14**, (2*S*)-3-*O*-D-glucopyranosyl-2-phenylpropionic acid and **15**,(2*R*)-3-*O*-β-D-glucopyranosyl-2-phenylpropionic acid. The configurations at C-2 of **11** and **12**, and **14** and **15** may be reversed. Compound **13** may be formed from **11** and **12** by acyl migration without enzymic reaction in the same manner as that which occurs on administration of 2-phenylpropionic acid [27].

Biotransformation of (RS)-2-(3-*benzoylphenyl*)*propionic acid* (**16**)

Compounds **17** and **18** were isolated as conversion products from (*RS*)-2-(3-benzoylphenyl)propionic acid (**16**) (Fig. 3). The FAB MS spectrum of **17** showed a peak at *m/z* 439 $[M+Na]^+$. The NMR spectra of the sugar moiety of **17** was in agreement with that of **3**. The FABMS spectrum of **18** showed a peak at *m/z* 601 $[M+Na]^+$ which was higher by 162 mass units than that of **17**, indicating the presence of an additional hexose unit in

Table 1. ^{13}C NMR data for compounds **2**–**5**, **7**, **9**–**15**, **17**, **18**, **20** and **21** (75 MHz, CD_3OD)

C	**2**	**3**	**4**	**5**		**7**	**9**	**11**	**12**	**13**				**14**	**15**	**17**		**18**		**20**	**21**
1	175.7	175.8	178.8	175.4		176.7	176.1	173.3	173.2	174.3	174.1	173.8	173.6	176.5	176.5	174.8	174.7	174.7	174.6	175.4	175.3
2	46.0	46.1	46.2	46.1		46.2	41.4	56.3	56.1	56.6	56.5	56.0		54.0	53.7	46.7	46.6	46.6	46.6	46.8	46.7
3	19.7	19.5	19.4	19.6	19.4	19.3	130.3	65.6	65.5	65.7	65.6			72.8	72.6	19.4		19.3		19.5	19.6
4	132.6	132.6	136.7	135.8		136.3	131.6	137.2	137.2	137.9	137.8	137.7	137.6	137.9	138.1	142.3		142.6	142.3	136.9	137.2
5	130.0	130.0	129.8	130.0	129.8	129.7	118.1	130.0[a]	130.1[b]	130.0[c]	129.9[c]			130.0[d]	130.0[e]	131.4		131.4		130.5	130.6
6	116.6	116.6	118.2	118.3	118.1	118.2	158.4	129.7[a]	129.8[b]	129.8[c]	129.7[c]			129.5[d]	129.6[e]	139.0		139.0		130.7	130.7
7	157.9	157.9	158.4	158.6	158.4	158.5	118.1	129.0	129.0	128.9	128.8			128.9	128.9	129.9[f]		129.9[g]		128.6[i]	128.6[j]
8	116.6	117.6	118.2	118.3	118.1	118.2	131.6	129.7[a]	129.8[b]	129.8[c]	129.7[c]			129.5[d]	129.6[e]	129.9[f]		129.9[g]		106.8	106.9
9	130.0	130.0	129.8	130.0	129.8	129.7		130.0[a]	130.1[b]	130.0[c]	129.9[c]			130.0[d]	130.0[e]	133.6	133.4	133.5		159.5	159.5
10																198.6		198.8	198 7	120.2	120.3
11																139.4		139.3	139.2	135.6	135.6
12																130.7	130.6	130.9	130.7	127.7[i]	127.7[j]
13																129.9[f]		129.9[g]		127.6	127.7
14																134.2		134.2	134.1		
15																130.1[f]		130.1[g]	130.0[g]		
16																130.3[f]	130.2[f]	130.3[g]	130.2[g]		
1′	96.2	96.2	102.6	96.3		102.6	102.6	96.5	96.4	91.5	91.3	96.5		105.3	104.8	96.4		94.8		96.4	94.5
2′	74.3	74.2	75.2	75.3		75.2	75.2	74.2	74.2	76.3	76.2	77.4	77.3	75.3	75.2	74.2	74.2	82.6	81.0	74.2	82.5
3′	78.3	78.4	78.2	78.4		78.2	78.3	78.3	78.3	72.4	72.3	76.4	76.3	78.3	78.3	78.4	78.3	79.1		78.3	79.2
4′	71.1	71.3	71.7	71.2		71.6	71.7	71.4	71.1	72.3	72.1	72.1	72.0	71.9	71.8	71.2		71.0[h]	70.9[h]	71.2	70.9[k]
5′	79.1	79.1	78.4	79.2		78.4	78.4	79.2	79.2	73.3	73.2	78.5		78.3	78.3	79.2		78.6	78.4	79.2	78.7
6′	62.4	62.6	62.8	62.5		62.8	62.8	62.7	62.4	62.9		63.0		63.1	63.0	62.6	62.5	62.5	62.4	62.4	62.4
1″				102.7	102.6													105.8	104.9		105.8
2″				75.2														76.3	76.1		76.4
3″				78.3														78.4	78.1		78.2
4″				71.7														71.8[h]			71.9[k]
5″				78.4														78.1			78.2
6″				62.8														63.1			63.3
OCH_2						62.1															
CH_3						14.7															
OCH_3																				56.0	56.0

[a–k] Assignments are interchangeable.

For **5**, **13**, **17** and **18**, there are several columns for each carbon because **5** is a mixture of diastereoisomers on the (*RS*)-2-(4-hydroxyphenyl)propionyl group at C-2, **17** and **18**, are mixtures of diastereoisomers on the (*RS*)-2-(3-benzoylphenyl)propionyl group at C-2, and **13** is a mixture of α- and β-anomers of the glucose moiety and diastereoisomers on the tropoyl group at C-2.

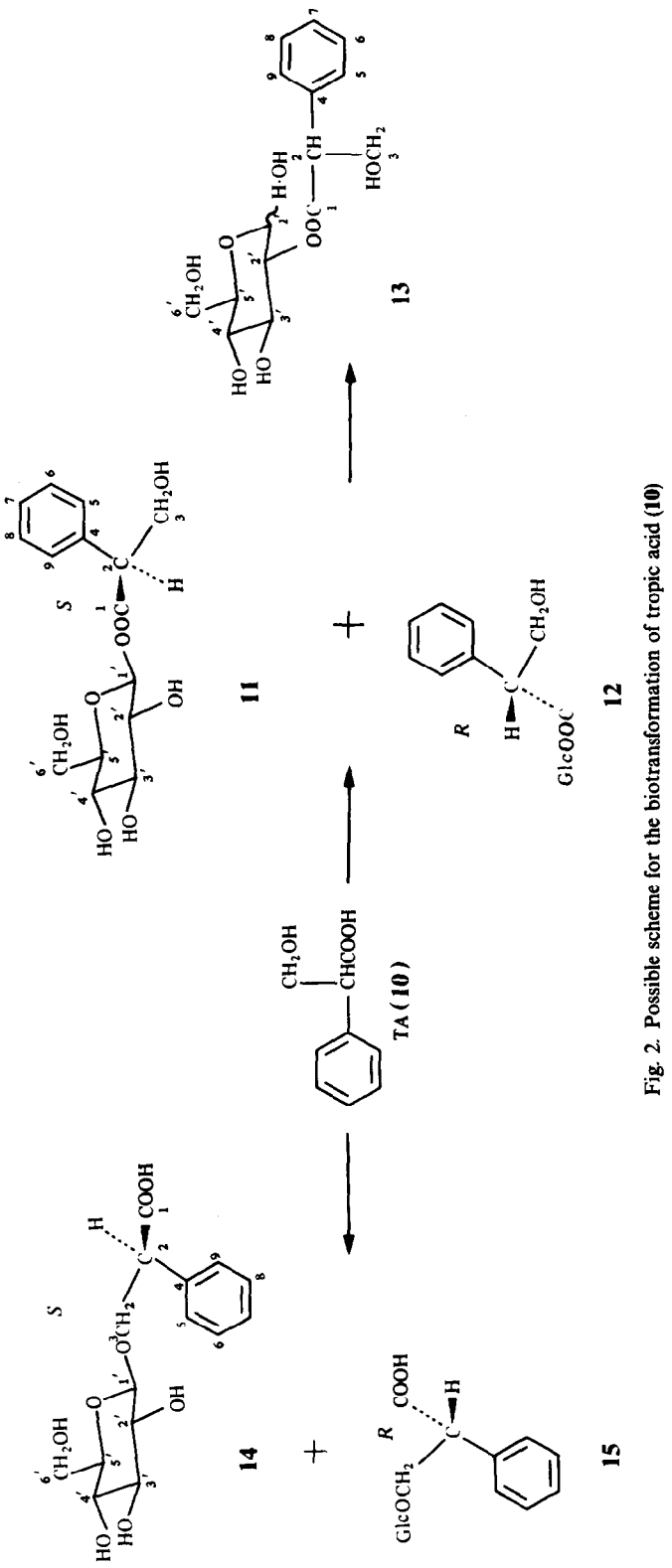

Fig. 2. Possible scheme for the biotransformation of tropic acid (10)

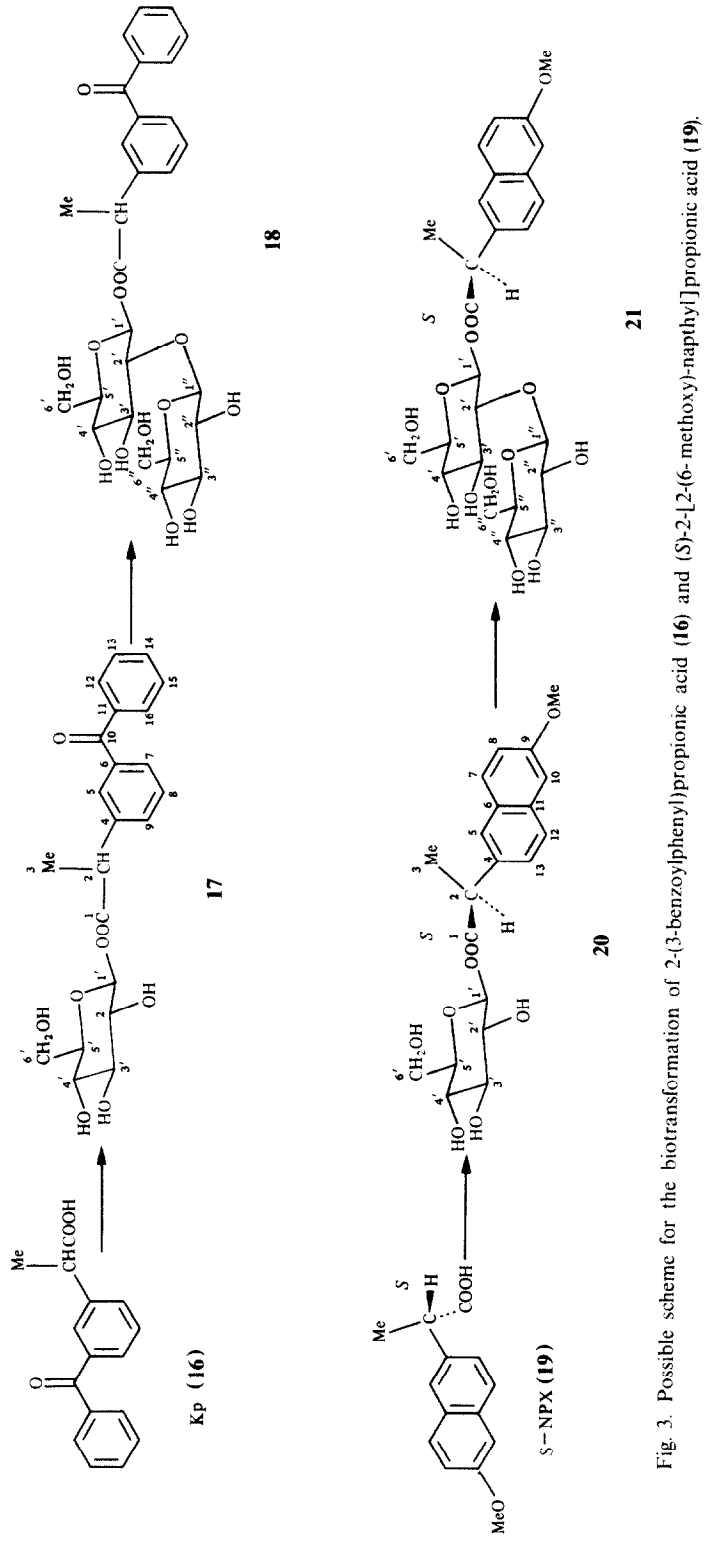

Fig. 3. Possible scheme for the biotransformation of 2-(3-benzoylphenyl)propionic acid (**16**) and (*S*)-2-[2-(6-methoxy)-napthyl]propionic acid (**19**).

17. In the NMR spectra of **18**, the chemical shifts and the coupling constants of the sugar moiety suggested the presence of two molecules of glucose. However, an acylation shift (*ca* $\delta+1.1$) for the anomeric proton of one glucose moiety suggested that the 2-(3-benzoylphenyl)propionyl group was attached to the anomeric position of a glucose residue. Glycosylation shifts (about δ-2, +8 and +9, respectively) of the anomeric carbon and the C-2 of the inner glucose and the anomeric carbon of the additional glucose moiety, respectively, suggested the additional glucose to be attached to the C-2 position of the inner glucose. The ^{13}C NMR spectrum of the sugar moiety of **18** was in good agreement with that of sophorose, which can be considered as an acylation product of the anomeric position of the inner glucose [32, 33, 35]. From these results **17** was assigned the structure of (2*RS*)-2-(3-benzoylphenyl)propionyl β-D-glucopyranoside and **18**, (2*RS*)-2-(3-benzoylphenyl) propionyl 2-*O*-β-D-glucopyranosyl-β-D-glucopyranoside.

Biotransformation of (*S*)-2-[2-(6-*methoxy*)-*naphthyl*]-*propionic acid* (**19**)

Compounds **20** and **21** were isolated as conversion products of (*S*)-2-[2-(6-methoxy)-naphthyl]propionic acid (**19**) (Fig. 3). The FABMS spectrum of **20** showed a peak at *m/z* 415 $[M+Na]^+$. In the NMR spectra of **20**, the chemical shifts and coupling constants of the sugar moiety of **20** were in agreement with that of **17**. The FABMS spectrum of **21** showed a peak at *m/z* 577 $[M+Na]^+$, which was larger by 162 mass units than that of **20**. In the NMR spectra of **21**, the chemical shifts and coupling constants of the sugar moiety were in agreement with those of **18**. From these results **20** was assigned the structure of (2*S*)-2-[2-(6-methoxy)-naphthyl]propionyl β-D-glucopyranoside and **21**, (2*S*)-2-[2-(6-methoxy)-naphthyl]propionyl 2-*O*-β-D-glucopyranosyl-β-D-glucopyranoside.

DISCUSSION

In the glycosylation of aromatic carboxylic acids by root culture of *P. ginseng*, the conversion ratios of 2-(4-hydroxyphenyl)propionic acid (**1**), ethyl 2-(4-hydroxyphenyl)propionate (**6**), (4-hydroxyphenyl)acetic acid (**8**), tropic acid (**10**), 2-(3-benzoylphenyl)propionic acid (**16**) and (*S*)-2-[2-(6-methoxy)-naphthyl]propionic acid (**19**) were 52.4, 22.4, 13.2, 28.8, 100.0 and 76.0%, respectively, after a three day incubation period (Table 2). The conversion ratio from **16** reached 100% as did that of 2-phenylpropionic acid in a previous study [27]. However, in the carboxylic acids, the conversion ratio of **1**, **8** and **10** were lower than that of **16**. The differences in conversion ratios possibly reflect the properties of the substrates, in particular, their polarity. The values such as inorganisity value, organisity/inorganisity value [38] and on HPLC all relate to polarity. When the relationships between these values, the biotransformation of aromatic carboxylic acids and the excretion of conversion products were investigated, it was observed that R_t of substrates on reversed phase HPLC correlated well to the degree of biotransformation and of excretion. The RR_t (column, Senshu Pak ODS-4301-N; benzoic acid = 1.00) of **1**, **5**, **8**, **10**, **16**, **19** and 2-phenylpropionic acid were 0.67, 1.54, 0.55, 0.64, 3.25, 4.02 and 1.31, respectively,and hence, as the free acids, the polarity of **8**, **10**, **1**, 2-phenylpropionic acid, **16** and **19** possibly decrease in the same order. The relationship between conversion ratio and RR_t is shown in Fig. 4. The conversion ratios for the substrates having a smaller R_t than 2-phenylpropionic acid were lower than that of 2-phenylpropionic acid (Fig. 4 and Table 2). From these results, it was clearly shown that the conversion ratio of a substrate is inversely related to its polarity. The lower conversion ratios of the more polar substrates may be due to the following. Glycosylation generally seems to be the mechanism by which plant cells decrease the toxicity of substrates. Because of their larger polarity, these substrates may be absorbed more slowly into the roots and may be less toxic for the root culture than 2-phenylpropionic acid. Thus glycosylation will proceed more slowly and give rise to a lower conversion ratio. Although the R_t of **19** was smaller than **16**, the conversion ratio was less than 100%. This may be because the concentration of **19** used (100 mg/l) was toxic to the root culture of *P. ginseng*. In appearance, the roots were slightly whitened and the medium was slightly reddened by the administration of **19**. In the root culture of *P. ginseng*, the conversion ratio from substrates having a smaller polarity such as 2-phenylpropionic acid and **16** may reach about 100% if the substrates have no specific toxicity and are easily metabolized.

Sugars binding to the administered substrates were glucose and/or sophorose which is β (1→2) linked glucobiose (Figs 1–4). It has been reported for biotransformations by plant cell cultures that gentiobiose, as a disaccharide, is the main sugar to be attached to administered substrates [13, 23, 24] Therefore the attachment of sophorose is a rare reaction. The plants [31–34] or the callus and root cultures [35–37] of *P. ginseng* produce the ginsenosides (Rb_1, Rb_2, Rc. Rd and Rf), pharmacologically active saponins, which contain sophorose as a constituent sugar. Therefore the root culture of *P. ginseng* used in this experiment also have ability to bind sophorose to administered substrates. On the other hand, in the biotransformation of 2-phenylpropionic acid, either glucose or primeverose, which is a disaccharide composed of glucose and xylose, was attached to the substrate. On the

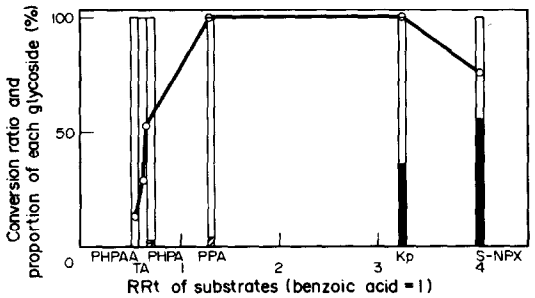

Fig. 4. Conversion ratio from each substrate and the proportion of conversion products binding each sugar: ⊖—⊖, conversion ratio; binding sugar: □ glucose; ⊞ glucose × 2; ▨ primeverose; ■ sophorose; RR_t of substrates (benzoic acid = 1, HPLC: Senshu Pak ODS-4301-N; with MeOH–H_2O, 1:1); PHPAA (**8**), (4-hydroxyphenyl)acetic acid; TA (**10**), tropic acid; PHPA (**1**), 2-(4-hydroxyphenyl)propionic acid; PPA,2-phenylpropionic acid (see Ref. 27); Kp (**16**), 2-(3-benzoylphenyl)propionic acid; S-NPX (**19**), (*S*)-2-[2-(6-methoxy)-naphthyl]propionic acid.

Table 2. Conversion ratio from each substrate and excretion ratio of the conversion products

Substrates	Products	Conversion ratio (%) R	M	T	Excretion ratio (M/T, %)
PHPA (**1**)	**2**	21.3	1.0	22.3	4
	3	20.9	0.7	21.6	3
	4	7.0	–	7.0	0
	5	1.4	0.1	1.5	7
	total	50.6	1.8	52.4	3
PHPA-E (**6**)	**7**	17.6	4.3	21.9	20
	2+3	0.5	–	0.5	0
	total	18.1	4.3	22.4	19
PHPAA (**8**)	**9**	13.2	–	13.2	0
Tropic acid (**10**)	**11**	5.3	0.3	5.6	5
	12	7.1	0.2	7.3	3
	13	4.5	0.7	5.2	13
	14+15	10.7	–	10.7	0
	total	27.6	1.2	28.8	4
Kp (**16**)	**17**	20.4	43.6	64.0	68
	18	29.7	6.3	36.0	18
	total	50.1	49.9	100.0	50
S-NPX (**19**)	**20**	9.2	24.6	33.8	73
	21	4.2	38.0	42.2	90
	total	13.4	62.6	76.0	82
PPA*	**22**	38.7	50.6	89.3	56
	23	0.9	5.6	6.5	86
	24	4.3	–	4.2	0
	total	43.8	56.2	100.0	56

R, in the root; M, in the medium; T, R + M.

PHPA (**1**), 2-(4-hydroxyphenyl)propionic acid; PHPA-E (**6**), ethyl 2-(4-hydroxyphenyl)propionate; PHPAA (**8**), (4-hydroxyphenyl)acetic acid; Kp (**16**), 2-(3-benzoylphenyl)propionic acid; S-NPX (**19**), (*S*)-2-[2-(6-methoxy)-naphthyl]propionic acid; PPA, 2-phenylpropionic acid; **22**, glucosyl ester of PPA; **23**, 2-*O*-(2-phenylpropionyl)-D-glucose; **24**, primeverosyl ester of PPA.

*See ref. [27] on the biotransformation of PPA.

administration of **1**, **8** and **10**, only glucose was attached to the substrates and **1** was further converted into a compound binding glucose at different sites. Compounds **16** and **19** were converted into sophorosyl esters, in addition to glucosyl esters. The proportion of sophorosyl esters in the total conversion products of **16** and **19** were 36.0 and 42.2%, respectively. The proportion of the sophorosyl ester of **19**, having a smaller R_f than that of **16**, was higher than that of **16**. These results indicated that the kinds and the proportion of sugars binding to substrates also depend primarily on their polarity, and the cultured roots convert substrates into the sugar esters and/or glycosides having a larger polarity and a lower toxicity. If a substrate having a smaller polarity than that of **19** is administered to root cultures of *P. ginseng*, the proportion of compounds binding sophorose would be high and would form a trisaccharide derivative. (*RS*)-2-(4-hydroxyphenyl)Propionic acid (**1**) and its ethyl ester (**6**), (*RS*)-tropic acid (**10**) and (*RS*)-2-(3-benzoylphenyl)propionic acid (**16**) among the substrates used in this experiment have asymmetric centres at C-2. In the biotransformation of **1**, **10** and **16**, the conversion of **1** into its glucosyl esters (**2** and **3**) was not selective with regard to the configuration at C-2 of **1**, and the glycosylation of **10** and **16** was also not selective. However, the configuration at C-2 of **4**, a glucoside of **1**, was *R* and the glucoside of (*S*)-2-(4-hydroxyphenyl)propionic acid has not been observed. The glucoside of the *R* configuration was dominantly produced, in the conversion of (*RS*)-ethyl 2-(4-hydroxyphenyl)propionate (**6**) into its glucoside (**7**). On glucosylation of (*RS*)-2-(4-hydroxyphenyl)propionic acid (**1**) at its phenolic hydroxy group, the root culture of *P. ginseng* selectively converted the free acid of the *R* configuration into its glucoside (**4**), and with its ethyl ester (**6**) showed somewhat less selectivity by producing a small amount of the glucoside with *S* configuration. The ratio of *R*- to *S*-**5** (4:1) was similar to that of **7**, and hence the ratio on the configuration between *R* and *S* may be about 4:1 for the conversion from the ester form of (*RS*)-2-(4-hydroxyphenyl)propionic acid (**1**). Therefore, **5** may have been produced from the glucosyl esters (**2** and **3**), instead of the glucoside (**4**), by the attachment of glucose at the phenolic hydroxy group.

When the root culture of *P. ginseng* was administered,

1 having both carboxylic and phenolic hydroxy groups or **10** having both carboxylic and alcoholic hydroxy groups, each substrate was converted into compounds binding glucose at the alcoholic or phenolic hydroxy and/or carboxylic groups, respectively. These results showed that the root culture has glucosylation ability to all those groups. The proportion of glucose esters (**2**, **3**, **11**–**13**) was higher than that of glucosides (**4**, **14** and **15**) on administration of **1** and **10**. On the other hand, on administration of **6**, de-ethylation followed by formation of glucosyl esters (**2** and **3**) occured to some extent, and almost all the products were phenolic glucosides. In general, the glycosylating selectivity to the group was low except for **8** which was selectively converted into the glucoside (**9**) and not glucosyl ester.

The excretion ratio of conversion products into the medium are shown in Table 2. The largest amounts of conversion products were excreted on administration of **16** and **19**, however, on administration of **1** and **10**, the excretion ratio were low, and the free acid form products (**4**, **9**, **14** and **15**) from **1**, **8** and **10** were not excreted. Although the disaccharide ester of 2-phenylpropionic acid was not excreted as described in an earlier paper [27] and that of **16** was slightly excreted and that of **19** in large amount. The excretion ratio of glucosyl esters formed from each substrate decreased in proportion to the quotient of the M_r divided by the RR_t of the substrate on reversed phase HPLC (Fig. 5). However, a relationship between excretion ratio and conversion products has not been observed. The suspension cultures of *Dioscoreophyllum cumminsii* and *Nicotiana tabacum* [25] which converted 2-phenylpropionic acid into its glucosyl ester in the same way as the root culture of *P. ginseng* did not excrete the product into the medium. Therefore, the excretion of conversion products depends firstly on plant species and/or cell strains, and, secondly, on properties of the substrates such as polarity and molecular size, that is, the excretion ratio of products was larger for the substrate having a low polarity and molecular size.

This experiment showed that, in the biotransformation of aromatic carboxylic acids by the root culture of *P. ginseng*, conversion ratio, the kinds and the proportion of binding sugars and the excretion of conversion products relate to the properties of substrates, in particular, the polarity.

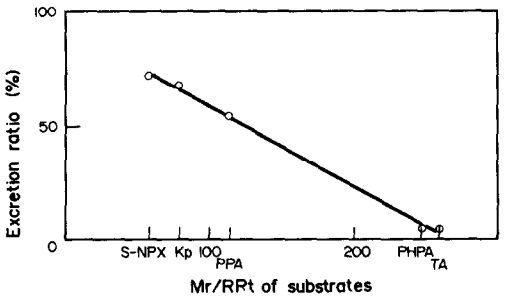

Fig. 5. Relationship between the excretion ratio of glucosyl esters and the M_r and RR_t of each substrate: RR_t (benzoic acid = 1, HPLC: Senshu Pak ODS-4301-N with MeOH–H_2O, 1:1).

EXPERIMENTAL

Mps: uncorr. NMR: 300 or 400 (CD_3OD). FAB-MS: JEOL JMS D-300 equipped with a direct inlet system.

Administration method. The root culture of *Panax ginseng* (Pg-1) [27, 28], 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, was transferred to liquid medium and cultured on a rotary shaker at 145 rpm and 25° in the dark. After three weeks, the test substrates, after being dissolved in H_2O, 50% EtOH or EtOH, were added to the subspension cultures and cultured for three days. The substrates used in this experiments were (4-hydroxyphenyl)acetic acid (colourless plates, mp 149–151°), (*RS*)-ethyl 2-(4-hydroxyphenyl)propionate (colourless liquid, bp 110–117°/5 mmHg), (*RS*)-2-(3-benzoylphenyl)propionic acid (white powder), (*S*)-2-[2-(6-methoxy)-naphthyl]propionic acid (white powder), supplied by Nissan Chemical Industries Ltd., (*RS*)-2-(4-hydroxyphenyl)propionic acid, prepared from (*RS*)-ethyl 2-(4-hydroxyphenyl)propionate by KOH hydrolysis, and (*RS*)-tropic acid purchased from Wako Pure Chemical (Osaka).

Isolation of conversion products. The root cultures previously administered the test substrates were separated into media and roots (about 30 g) by filtration through Nylon cloth. The roots were homogenized in MeOH and the homogenate filtered. The filtrate was concd, dissolved in H_2O and combined with the medium. The soln was applied to a column of Diaion HP-20 and washed with water followed by elution with MeOH. From the MeOH eluates, the conversion products were isolated by a combination of silica gel CC, HPLC and/or crystallization. The eluents used for silica gel CC were $CHCl_3$–MeOH–H_2O (25:5:3, lower layer, for **11**–**15**, 38:12:5 for **2**–**5** and 42:8:5 for **7**) and $CHCl_3$–MeOH (15:1 for **17** and **18**, 9:1 for **20** and **21** and 4:1 for **9**). HPLC was carried out on a Senshu-Pak ODS-4301-N(300 × 10 mm). The eluents used were MeOH–H_2O(1:4 for **2**–**5**, **11**–**13**, 1:3 for **14** and **15** 2:3 for **7** and 1:1 for **17**, **18**, **20** and **21**. From these eluents, **10** was crystallized from MeOH and **9**, **14**, **20** and **21** from H_2O. Compound **15** was obtained from the mother liquid of **14**.

Quantitative analysis of conversion products. The media and MeOH extracts of the roots from 250 ml of suspension culture which had been incubated with 25 mg of test substrate 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 (1:4 for **2**–**5**, **9**, **11**–**15**, 2:3 for **7** and 1:1 for **17**, **18**, **20** and **21**), detection by differential refractometer and UV (254 nm) absorption.

(2S)-2-(4-*hydroxyphenyl*)*Propionyl* β-D-*glucopyranoside* (**2**). Amorphous solid: $[\alpha]_D^{22}$ +27.9° (MeOH; *c* 1.40); IR ν_{max}^{KBr} cm^{-1}: 3390, 1735, 1515; ^1H NMR (CD_3OD): δ 3.82 (1H, *q*, *J* = 7 Hz, H-2), 1.52 (3H, *d*, *J* = 7 Hz, H-3), 5.55 (1H, *d*, *J* = 8 Hz, H-1'), 3.87 (1H, *dd*, *J* = 12, 1.8 Hz) and 3.72 (1H, *dd*, *J* = 12, 4 Hz, H-6'), 7.19–7.23 (2H, *m*, H-5 and 9), 6.77–6.83 (2H, *m* H-6 and 8), 3.37–3.53 (4H, *m* H-2', 3', 4' and 5'); ^{13}C NMR (CD_3OD): see Table 1; FAB MS *m/z*: 351 $[M+Na]^+$; CD (EtOH; *c* 7.13 × 10^{-4}) $\Delta\varepsilon^{21}$ +3.15 (232) (pos. max).

(2R)-2-(4-*hydroxyphenyl*)*Propionyl* β-D-*glucopyranoside* (**3**). Amorphous solid: $[\alpha]_D^{22}$ −13.9° (MeOH; *c* 1.09); IR ν_{max}^{KBr} cm^{-1}: 3400, 1740, 1515; ^1H NMR (CD_3OD): δ 3.82 (1H, *q*, *J* = 7 Hz, H = 2), 1.55 (3H, *d*, *J* = 7 Hz, H-3), 5.54 (1H, *d*, *J* = 8 Hz, H-1'), 3.92 (1H, *dd*, *J* = 12, 2 Hz) and 3.77 (1H, *dd*, *J* = 12, 4.6 Hz, H-6'), 7.20–7.26 (2H, *m*, H-5 and 9), 6.79–6.84 (2H, *m*, H-6 and 8), 3.35–3.47 (4H, *m*, H-2', 3', 4' and 5'); ^{13}C NMR (CD_3OD): see Table 1; FABMS *m/z*: 351 $[M+Na]^+$; CD(EtOH; *c* 5.61 × 10^{-4}) $\Delta\varepsilon^{21}$ −2.70 (232) (neg. max).

(2R)-2-(4-*O*-β-D-*glucopyranosylphenyl*)*Propionic acid* (**4**). Amorphous solid: $[\alpha]_D^{22}$ −34.4° (MeOH; *c* 1.18); IR ν_{max}^{KBr} cm^{-1}: 3400, 1715, 1515; ^1H NMR (CD_3OD): δ 3.75 (1H, *q*, *J* = 7 Hz, H-2), 1.51 (3H, *d*, *J* = 7 Hz, H-3), 5.01 (1H, *d*, *J* = 8 Hz, H-1'), 3.98 (1H, *dd*, *J* = 12, 1.8 Hz) and 3.79 (1H, *dd*, *J* = 12, 5 Hz, H = 6'), 7.31–7.36 (2H, *m*, H-5 and 9), 7.12–7.17 (2H, *m*, H-6 and 8), 3.48–3.58 (4H, *m*, H-2', 3', 4' and 5'); ^{13}C NMR (CD_3OD): see Table 1; FAB MS *m/z*: 351 $[M+Na]^+$; CD (EtOH; *c* 6, 10 × 10^{-4} $\Delta\varepsilon^{22}$ − 1.74 (220) (neg. max).

(2RS)-2-(4-*O*-β-D-*glucopyranosylphenyl*)*Propionyl* β-*glucopyranoside* (**5**). Amorphous solid: $[\alpha]_D^{24}$ −28.4° (MeOH; *c* 0.55; IR ν_{max}^{KBr} cm^{-1}: 3380, 1740 1515; ^1H NMR (CD_3OD): δ 3.88 (1H, *q*, *J* = 7 Hz, H-2), 1.55 (2.4 H, *d*, *J* = 7 Hz) and 1.51 (0.6 H, *d*, *J* = 7 Hz, H-3), 5.56 (1H, *d*, *J* = 8 Hz, H-1'), 3.87 (1H, *dd*, *J* = 12, 2 Hz) and 3.72 (1H, *dd*, *J* = 12, 4 Hz, H-6'), 4.93 (1H, *d*, *J* = 8 Hz, H = 1''), 3.98 (1H, *dd*, *J* = 12, 2 Hz), and 3.79 (1H, *dd*, *J* = 12, 5 Hz, H-6''), 7.32–7.37 (2H, *m* H-5 and 9), 7.12–7.17 (2H, *m*, H-6 and 8), 3.36–3.56 (8H, *m*, H-2', 2'', 3', 3'', 4', 4'', 5' and 5''); ^{13}C NMR (CD_3OD): see Table 1; FABMS *m/z*: 513 $[M+Na]^+$.

(2RS)-*Ethyl* 2-(4-*O*-β-D-*glucopyranosylphenyl*)*propionate* (**7**). Amorphous solid: $[\alpha]_D^{19}$ −51.3° (MeOH; *c* 0.99); IR ν_{max}^{KBr} cm^{-1}: 3420, 1730, 1510; ^1H NMR (CD_3OD): δ 3.78 (1H, *q*, *J* = 7 Hz, H-2), 1.51 (3H, *d*, *J* = 7 Hz, H-3), 4.99 (1H, *d*, *J* = 7.4 Hz, H-1'), 3.98 (1H, *dd*, *J* = 12, 2 Hz) and 3.79 (1H, *dd*, *J* = 12, 5 Hz, H-6'), 4.09 and 4.07 (each 0.8H, *q*, *J* = 7.2Hz and 4.11) and 4.05 (each 0.2H, *q*, *J* = 7.2 Hz, OCH_2), 1.17 (3H, *t*, *J* = 7.2 Hz, Me), 7.28–7.33 (2H, *m*, H-5 and 9), 7.11–7.16 (2H, *m*, H-6 and 8), 3.46– 3.57 (4H, *m*, H-2', 3', 4' and 5'); ^{13}C NMR (CD_3OD): see Table 1: FAB MS *m/z*: 379 $[M+Na]^+$ CD (EtOH; *c* 5.06 × 10^{-4}) $\Delta\varepsilon^{21}$ − 2.16 (224) (neg. max).

(4-O-β-D-*glucopyranosylphenyl*)*Acetic acid* (**9**). Colourless needles: mp 185–186°; $[\alpha]_D^{24}$ −51.9° (MeOH, *c* 1.14); IR ν_{max}^{KBr} cm^{-1}: 3370, 1715, 1520; ^1H NMR (CD_3OD): δ 3.63, 2H (*s*, H-2), 4.98 (1H, *d*, *J* = 7.6 Hz, H-1'), 3.98 (1H, *dd*, *J* = 12, 3 Hz) and 3.79 (1H, *dd*, *J* = 12, 5 Hz, H-6'), 7.26–7.33 (2H, *m*, H-4 and 8), 7.11–7.17 (2H, *m*, H-5 and 7), 3.48–3.58 (4H, *m*, H-2', 3', 4' and 5'); ^{13}C NMR (CD_3OD): see Table 1; FAB MS *m/z*: 337 $[M+Na]^+$.

(2S)-3-*hydroxy*-2-*Phenylpropionyl* β-D-*glucopyranoside* (**11**). Colourless needles: mp 150–151°; $[\alpha]_D^{24}$ −4.1° (MeOH; *c* 1.18); IR ν_{max}^{KBr} cm^{-1}: 3420, 1745; ^1H NMR (CD_3OD): δ 3.88 (1H, *dd*, *J* = 9, 5.5 Hz, H-2), 4.11 (1H, *dd*, *J* = 11, 9 Hz) and 3.77 (1H, *dd*, *J* = 11, 5.5 Hz, H-3), 5.51 (1H, *d*, *J* = 8 Hz, H-1'), 3.82 (1H, *dd*, *J* = 12, 2 Hz) and 3.36 (1H, *dd*, *J* = 12, 5.4 Hz, H-6'), 7.24–7.36 (5H, *m*, H = 5, 6, 7, 8 and 9), 3.32–3.42 (4H, *m*, H-2', 3', 4' and 5'); ^{13}C NMR (CD_3OD): see Table 1; FABMS *m/z*: 351 $[M+Na]^+$; CD (EtOH; *c* 6.34 × 10^{-4}) $\Delta\varepsilon^{19}$ − 1.91 (218) (neg. max).

(2R)-3-*hydroxy*-2-*Phenylpropionyl* β-D-*glucopyranoside* (**12**). Amorphous solid: $[\alpha]_D^{23}$ + 22.4° (MeOH; *c* 1.32); IR ν_{max}^{KBr} cm^{-1}: 3405, 1745; ^1H NMR (CD_3OD): δ 3.87 (1H, *dd*, *J* = 9, 5.5 Hz, H-2), 4.10 (1H, *dd*, *J* = 10.5, 9 Hz) and 3.74 (1H, *dd*, *J* = 10.2, 5.5 Hz, H-3), 5.53 (1H, *d*, *J* = 8 Hz, H-1'), 3.77 (1H, *d*, *J* = 11.5, 2 Hz) and 3.63 (1H, *dd*, *J* = 11.5, 4 Hz, H-6'), 7.33–7.45 (5H, *m*, H-5, 6, 7, 8 and 9) 3.28–3.45 (4H, *m*, H-2', 3', 4' and 5'); ^{13}C NMR (CD_3OD): see Table 1; FAB MS *m/z*: 351 $[M+Na]^+$; CD (EtOH; *c* 5.85 × 10^{-4}) $\Delta\varepsilon^{19}$ + 3.83 (219) (pos. max).

(2RS)-2-O-(3-*hydroxy*-2-*phenylpropionyl*)-D-*Glucose* (**13**). Amorphous solid: $[\alpha]_D^{18}$ + 53.2° (MeOH; *c* 0.77); IR ν_{max}^{KBr} cm^{-1}: 3395, 1730; ^1H NMR CD_3OD): δ 5.38 (0.3H, *d*, *J* = 3.6 Hz), 5.28 (0.3H, *d*, *J* = 3.6 Hz), 4.76 (0.2H, *d*, *J* = 8 Hz) and 4.60 (0.2H, *d*, *J* = 8 Hz, H-1'), 4.73 (0.6H, *dd*, *J* = 10, 3.6 Hz) and 4.82 (0.4H, *dd*, *J* = 9.5, 8 Hz, H-2'), 3.62 (0.2H, *dd*, *J* = 9.5, 9 Hz) and 3.56 (0.2H, *dd*, *J* = 9.5, 9 Hz, H-3'), 3.50 (0.3H, *t*, *J* = 9 Hz), 3.46 (0.3H, *t*, *J* = 9 Hz), 3.47 (0.2H, *t*, *J* = 9 Hz) and 3.43 (0.2H, *t*, *J* = 9 Hz, H-4'), 3.38 (0.2H, *ddd*, *J* = 9, 5.5, 2 Hz) and 3.37 (0.2H, *ddd*, *J* = 9, 5.5, 2 Hz, H-5'), 7.22–7.42 (5H, *m*, H-5, 6, 7, 8 and 9), 4.17–4.23 (1H, *m*, H-3), 3.72–4.02 (4.6H, *m*, H = 2, 3, 3', 5' and 6'); ^{13}C NMR (CD_3OD): see Table 1; FABMS *m/z*: 351 $[M+Na]^+$.

(2S)-3-O-β-D-*Glucopyranosyl*-2-*phenylpropionic acid* (**14**). Colourless needles: mp 85–86°; $[\alpha]_D^{21}$ + 48.8° (MeOH; *c* 1.38); IR ν_{max}^{KBr} cm^{-1}: 3410, 1725; ^1H NMR (CD_3OD): δ 4.46 (1H, *d*, *J* = 7.8 Hz, H-1'), 3.26 (1H, *dd*, *J* = 9, 7.8 Hz, H-2'), 3.96 (1H, *dd*, *J* = 11.5, 2 Hz) and 3.75 (1H, *dd*, *J* = 11.4, 5 Hz, H-6'), 4.03–4.13 and 4.23–4.32 (total 3H, *m*, H-2 and 3), 7.32–7.47 (5H, *m*, H = 5, 6, 7, 8 and 9), 3.35–3.48 (3H, *m*, H-3', 4' and 5'); ^{13}C NMR (CD_3OD): see Table 1; FABMS *m/z*: 329 $[MH]^+$; CD (EtOH, *c* 7.50 × 10^{-4}) $\Delta\varepsilon^{19}$ − 4.12 (218) (neg. max).

(2R)-3-O-β-D-*Glucopyranosyl*-2-*phenylpropionic acid* (**15**). Amorphous solid: $[\alpha]_D^{24}$ + 8.2° (EtOH; *c* 0.39); IR ν_{max}^{KBr} cm^{-1}: 3420, 1720; ^1H NMR: δ 3.84 (1H, *dd*, *J* = 9.5, 5 Hz, H-2), 4.56 (1H, *t*, *J* = 9.5 Hz) and 4.06 (1H, *dd*, *J* = 9.5, 5 Hz, H-3), 4.38 (1H, *d*, *J* = 7.8 Hz, H-1'), 3.27 (1H, *dd*, *J* = 9 Hz, 7.8 Hz, H-2'), 3.96 (1H, *dd*, *J* = 12, 2 Hz) and 3.76 (1H, *dd*, *J* = 12, 5 Hz, H-6'), 7.37–7.47 (5H, *m*, H-5, 6, 7, 8 and 9), 3.33–3.46 (3H, *m*, H-3', 4' and 5') ^{13}C NMR (CD_3OD): see Table 1; FABMS *m/z*: 351 $[M+Na]^+$; CD (EtOH; *c* 9.51 × 10^{-4}) $\Delta\varepsilon^{21}$ + 4.40 (218) (pos. max).

(2RS)-2-(3-*benzoylphenyl*)*Propionyl* β-D-*glucopyranoside* (**17**). Amorphous solid: ^1H NMR (CD_3OD): δ 4.04 (1H, *g*, *J* = 7 Hz, H-2), 1.64 and 1.63 (each 1.5H *d*, *J* = 7 Hz, H-3), 5.61 and 5.59 (each 0.5H, *d*, *J* = 7.5 Hz, H-1'), 3.87 (1H, *dd*, *J* = 12, 1.8 Hz) and 3.73 (1H, *dd*, *J* = 12, 4.5 Hz, H-6'), 7.84–7.90, 7.70–7.77 and 7.54–7.65 (total 9H, *m*, H-5, 7, 8, 9, 12, 13, 14, 15 and 16), 3.38–3.55 (4H, *m*, H-2', 3', 4' and 5''); ^{13}C NMR (CD_3OD); see Table 1; FABMS *m/z*: 439 $[M+Na]^+$.

(2RS)-2-(3-*benzoylphenyl*)*propionyl* 2-O-β-D-*Glucopyranosyl*-β-D-*glucopyranoside* (**18**). Amorphous solid: $[\alpha]_D^{20}$ + 2.2° (MeOH; *c* 0.81); IR ν_{max}^{KBr} cm^{-1}: 3405, 1750, 1660; ^1H NMR (CD_3OD): δ 4.03 and 3.97 (each 0.5H, *q*, *J* = 7 Hz, H-2), 1.52 and 1.48 (each 1.5H, *d*, *J* = 7 Hz, H-3), 5.60 and 5.57 (each 0.5H, *d*, *J* = 7.5 Hz, H-1'), 3.13 (1H, *ddd*, *J* = 9.5, 5, 2 Hz, H-5'), 4.56 and 4.53 (each 0.5H, *d*, *J* = 8 Hz, H-1'), 3.17 ajnd 3.03 (each 0.5H, *dd*, *J* = 9, 8 Hz, H-2''), 7.70–7.77, 7.57–7.66 and 7.41–7.52 (total 9H, *m*, H-5, 7, 8, 9, 12, 13, 14, 15 and 16), 3.49–3.85 (6H, *m*, H-2', 6', 5'' and 6''), 3.23–3.41 (4H, *m*, H-3', 4', 3'' and 4''); ^{13}C NMR (CD_3OD): see Table 1; FABMS *m/z*: 601 $[M+Na]^+$.

(2S)-2-(2-(6-*methoxy*)-*naphthyl*) *Propionyl* β-D-*glucopyranoside* (**20**). Colourless needles: mp 168–170°; $[\alpha]_D^{22}$ + 27.0° (MeOH, *c* 0.74); IR ν_{max}^{KBr} cm^{-1}: 3395, 1750; ^1H NMR (CD_3OD): δ 3.96 (1H, *q*, *J* = 7 Hz, H-2), 1.56 (3H, *d*, *J* = 7 Hz, H-3), 7.19 (1H, *d*, *J* = 2.5 H, H-8), 7.10 (1H, *dd*, *J* = 9, 2.5 Hz, H-10), 7.40(1H, *dd*, *J* = 8.5, 2 Hz, H-13), 5.51 (1H, *d*, *J* = 8 Hz, H-1'), 3.75 (1H, *dd*, *J* = 12, 2 Hz) and 3.61 (1H, *dd*, *J* = 12, 4.5 Hz, H-6'), 3.88 (3H, *s*, Me), 7.68–7.76 (3H, *m*, H-5, 7 and 12), 3.29–3.42 (4H, *m*, H-2', 3', 4' and 5'); ^{13}C NMR (CD_3OD): see Table 1; FABMS *m/z*: 415 $[M+Na]^+$; CD (EtOH; *c* 5.45 × 10^{-4}) $\Delta\varepsilon^{21}$ − 1.33 (218) (neg. max), + 2.22 (232) (pos. max).

(2S)-2-[2-(6-*methoxy*)-*naphthyl*] *Propionyl* 2-*O*-β-D-*glucopyranosyl*-β-D-*glucopyranoside* (**21**). Colourless needles: mp 215–217°; $[\alpha]_D^{24}$ + 11.7° (MeOH; *c* 0.41); IR ν_{max}^{KBr} cm^{-1}: 3405, 1750; ^1H NMR (CD_3OD): δ 4.16 (1H, *q*, *J* = 7 Hz, H-2), 1.65 (3H, *d*, *J* = 7Hz, H-3), 7.29 (1H, *d*, *J* = 2.5 Hz, H-8), 7.20 (1H, *dd*, *J* = 9, 2.5 Hz, H-10), 7.53 (1H, *dd*, *J* = 8.5, 2 Hz, H-13), 5.73 (1H, *d*, *J* = 7.8 Hz, H-1'), 3.85 (1H, *dd*, *J* = 11.5, 1.5 Hz, H-6'), 4.70 (1H, *d*, *J* = 8 Hz, H-1''), 3.33 (1H, *dd*, *J* = 9, 8 Hz, H-2''), 4.03 (1H, *dd*, *J* = 11.5, 1.5 Hz) and 3.82 (1H, *dd*, *J* = 11.5, 5 Hz, H-6''), 3.98 (3H, *s*, OMe), 7.77–7.84 (3H, *m*, H-5, 7 and 12), 3.66–3.74 (3H, *m*, H-2', 6' and 5''), 3.38–3.52 (5H, *m*, H-3', 4', 5'. 3'' and 4''); ^{13}C NMR (CD_3OD): see Table 1; FABMS *m/z*: 577 $[M+Na]^+$; CD (EtOH; *c* 3.57 × 10^{-4}). $\Delta\varepsilon^{21}$ − 5.43 (218) (neg. max), + 6.79 (232) (232) (pos. max).

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