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BIOTRANSFORMATION OF PHENYLCARBOXYLIC ACIDS BY PLANT CELL CULTURES*

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Key Word Index—*Aconitum japonicum*; *Coffea arabica*; *Dioscoreophyllum cumminsii*; *Glycyrrhiza echinata*; *Nicotiana tabacum*; cell suspension culture; biotransformation; hydroxylation; glycosylation; reduction.

Abstract—A suspension culture of *Glycyrrhiza echinata* converted benzoic acid into its glucosyl ester. Suspension cultures of *Aconitum japonicum*, *Coffea arabica*, *Dioscoreophyllum cumminsii* and *Nicotiana tabacum*, transformed benzoic acid into its gentiobiosyl ester in addition to the glucosyl ester. The suspension cultures of *A. japonicum* and *G. echinata* converted phenylacetic acid into the esters attached to the C-6 position of glucose, that is, 6-*O*-phenylacetyl-D-glucose and ethyl 6-*O*-phenylacetyl-β-D-glucopyranoside. That of *D. cumminsii* converted phenylacetic acid into the glucose ester and also into phenethyl β-D-glucopyranoside showing glucosylation after the reduction of the carboxylic group. These suspension cultures converted cinnamic acid into *p*-coumaric acid and its glucosyl ester and *p*-coumaric acid into its glucosyl ester. However, the conversion of caffeic acid was not observed. The suspension cultures of *A. japonicum* and *C. arabica* converted 3-phenylpropionic acid into its gentiobiosyl ester. On the other hand, the culture of *D. cumminsii* did not produce the glycosyl ester but instead 3-(4-hydroxyphenyl)propionic acid was formed, thus showing hydroxylation capability.

INTRODUCTION

We have investigated the biotransformation abilities of plant cell cultures to phenylpropanoides and their analogues. In our previous papers [1–5], we reported on the biotransformation of 2-phenylpropionic acid(2-PPA) and its derivatives such as 2-(4-hydroxyphenyl)propionic acid (PHPA) and tropic acid. The suspension culture of *Coffea arabica* [1, 2] mainly converted phenylacetic acid, 2-PPA, PHPA and tropic acid into their sucrose esters, that of *Aconitum japonicum* [3] converted 2-PPA into ethyl 6-*O*-(2-phenylpropionyl)-β-D-glucopyranoside and those of *Dioscoreophyllum cumminsii* and *Nicotiana tabacum* [3] converted 2-PPA into its glucosyl and gentiobiosyl esters. The present paper reports on the biotransformation of benzoic acid, phenylacetic acid, 3-phenylpropionic acid and naturally occurring phenylpropanes (*trans*-cinnamic acid, *trans*-*p*-coumaric acid and *trans*-caffeic acid) by the suspension cultures of *A. japonicum*, *C. arabica*, *D. cumminsii*, *Glycyrrhiza echinata* and *N. tabacum*. The biotransformation abilities of plant cell cultures to C_6–C_1, C_6–C_2 or C_6–C_3 skeleton substrates were investigated.

RESULTS

Biotransformation of benzoic acid

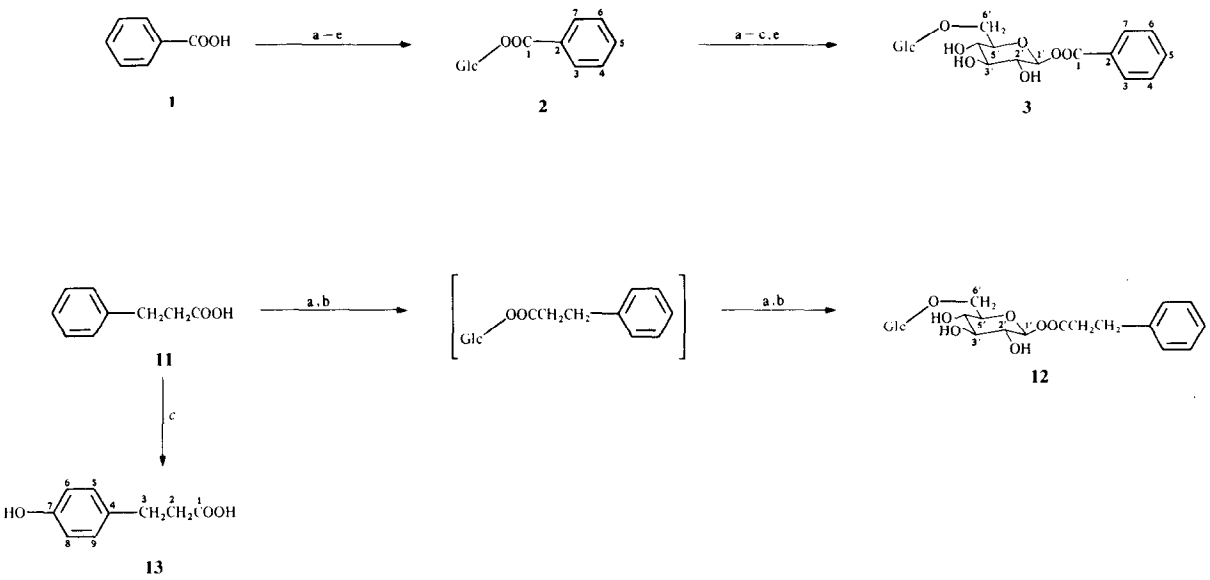
After the administration of benzoic acid (**1**) to the plant cell suspension cultures, the β-glucosyl ester (**2**) of **1** was isolated from cultured cells of *Glycyrrhiza echinata* by a combination method of silica gel chromatography and HPLC. From cultured cells of *Aconitum japonicum*, *Coffea arabica*, *Dioscoreophyllum cumminsii* and *Nicotiana tabacum* compound **3** in addition to **2** was isolated. The FABMS spectrum of **3** showed a peak at *m/z* 447 $[MH]^+$. The ^{13}C NMR spectrum of **3** exhibited signals showing the presence of an additional β-glucosyl unit (Table 1). The glycosylation shift at C-6 (δ +7.2) of the inner glucose moiety indicated that the terminal glucose in **3** is linked to the C-6 position of the inner glucose. The ^{13}C NMR spectrum of the sugar moiety of **3** was in good agreement with that of gentiobiose and the ^1H NMR spectrum (Experimental) also showed the structure of **3** to be the β-gentiobiosyl ester of **1**.

Biotransformation of phenylacetic acid

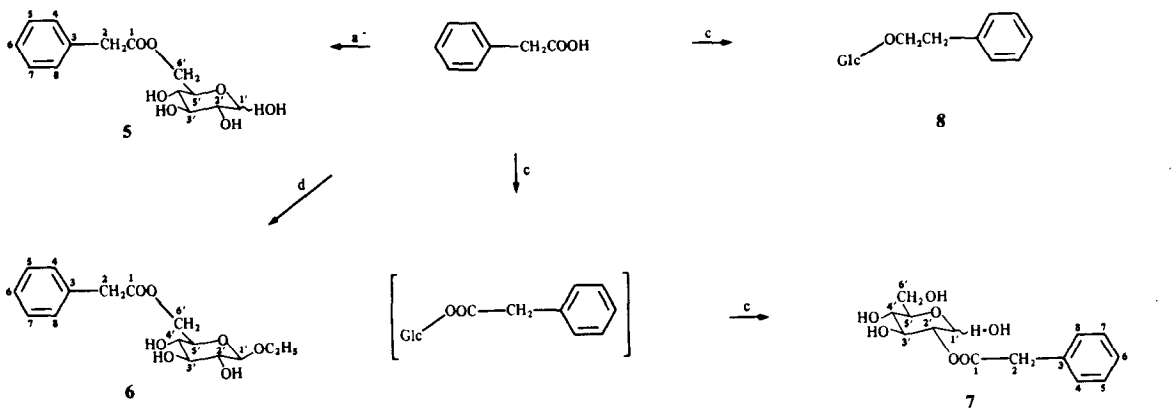
From the conversion products of phenylacetic acid (**4**), compound **5** was isolated from the suspension culture of *A. japonicum* and **6**, from the medium of *G. echinata*. The FABMS spectrum of **5** showed a peak at *m/z* 321 $[M+Na]^+$. In the ^1H and ^{13}C NMR spectra of **5**, there were signals showing the presence of the α- and β-anomers of glucose acylated at the C-6 position, in addition to those due to the phenylacetyl group. The FABMS spectrum of **6** showed a peak at *m/z* 349 $[M+Na]^+$ which was larger by 28 mass units than that of **5**. The ^1H and ^{13}C NMR spectra of **6** were in good agreement with those of the β-anomer of **5** excepting an additional set of signals due to the ethyl group carrying an oxygen function. In the ^{13}C NMR spectrum a glycosylation shift at the anomeric carbon of a glucose moiety was observed. This glycosylation shift showed the ethyl group to be attached to the anomeric position of the glucose moiety. From these results **5** was assigned the structure of 6-*O*-phenylacetyl-D-glucose and **6** was ethyl 6-*O*-phenylacetyl-β-D-glucopyranoside.

From the cultured cells of *D. cumminsii*, compound **7** and phenethyl β-D-glucopyranoside (**8**) [6] were isolated. The FABMS spectrum of **7** showed a peak at *m/z* 321 $[M+Na]^+$. The ^1H and ^{13}C NMR spectra of **7** exhibited the signals showing the presence of the α- and β-anomers of a

* Part 64 in the series of 'Studies on Plant Tissue Culture'. For part 63, see Ushiyama, M. and Furuya, T. (1989) *Phytochemistry* **28** (in press).



Scheme 1. Biotransformations of benzoic acid (**1**) and 3-phenylpropionic acid (**11**) by plant cell cultures. (a) *Aconitum japonicum*; (b) *Coffea arabica*; (c) *Dioscoreophyllum cumminsii*; (d) *Glycyrrhiza echinata*; (e) *Nicotiana tabacum*.



Scheme 2. Biotransformations of phenylacetic acid (**4**) by plant cell cultures. The suspension culture of *C. arabica* converted **4** into the sucrose ester, see ref. [1] (a–d). See legend to Scheme 1.

glucose moiety. The acylation shift (*ca* δ +1.2) for H-2′ of the glucose moiety indicated the phenylacetyl group to be attached to the C-2 position of the glucose moiety. Thus **7** may be 2-*O*-phenylacetyl-D-glucose. Although the glucosyl ester of **4** has not been isolated, compound **7** was transferred to the glucosyl ester of **4** by acyl migration. The biotransformation of **2** into **8** showed that the suspension culture of *D. cumminsii* reduced the carboxylic group of **2** to a primary alcohol. Although 2-phenylethanol and its glucoside (**8**) were identified from the suspension culture of *Rosa damascena* [7], these compounds were not identified in plant or suspension culture of *D. cumminsii*, which had no administration or was fed only ethanol.

Biotransformation of C_6–C_3 *compounds*

When *trans*-cinnamic acid (**9**) was incubated with the suspension culture of *N. tabacum*, the β-glucosyl ester of *p*-coumaric acid (**10**) [8, 9] was isolated from the cultured cells. *p*-Coumaric acid was not isolated from the suspension culture of *N. tabacum* previously administered cinnamic acid, but identified by TLC. From the suspension cultures of *A. japonicum*, *C. arabica*, *D. cumminsii* and *G. echinata* previously administered cinnamic acid, *p*-coumaric acid and its glucosyl ester (**10**) were identified by TLC. In the administration of *trans*-*p*-coumaric acid, although its conversion product was not isolated, the glucosyl ester of *p*-coumaric acid (**10**) was identified by TLC. When *trans*-caffeic acid was administered, caffeic acid was adsorbed by the cells but conversion was not observed.

When 3-phenylpropionic acid (**11**) was administered, compound 12 was isolated from the cultured cells of *A. japonicum* and *C. arabica*. The FABMS spectrum of **12** showed a peak at *m/z* 497 $[M+Na]^+$. The ^1H and ^{13}C NMR spectra exhibited the signals due to the 3-phenylpropionyl group and sugar moiety and the spectra

Table 1. ^{13}C NMR data for compounds **2**, **3**, **5**, **7**, **8**, **10**, **12**, **13**, **15** (75 or 100 MHz, CD_3OD) and **6** ($CDCl_3$)

C	2	3	5		6	7		8	10	12	13	15	
1	167.1	167.1	173.8		172.3	173.4	172.9	72.0	168.3	173.6	177.3	176.2	
2	135.0	135.1	42.1	42.0	41.2	42.2	41.9	37.5	114.3	37.1	37.5	47.0	46.9
3	131.1[a]	131.1[b]	135.9		133.7	140.1	139.0	140.4	148.6	31.8	31.6	19.5	19.4
4	131.2[a]	131.2[b]	130.8[e]		129.3[f]	130.6[g]		130.3[h]	127.2	142.1	133.3	142.2	
5	129.9	130.0	129.8[e]		128.6[f]	129.6[g]	129.5[g]	129.7[h]	131.7	129.8[i]	130.5	129.8[k]	129.7[k]
6	131.2[a]	131.2[b]	128.3		127.2	128.1	128.0	127.5	117.1	129.7[i]	116.5	128.7[k]	
7	131.1[a]	131.1[b]	129.8[e]		128.6[f]	129.6[g]	129.5[g]	129.7[h]	160.9	127.6	157.1	128.3	
8			130.8[e]		129.3[f]	130.6[g]		130.3[h]	117.1	129.7[i]	116.5	128.7[k]	
9									131.7	129.8[i]	130.5	129.8[k]	129.7[k]
1′	96.6	96.6	94.3	98.6	102.3	91.3	96.5	104.7	95.8	96.0		94.1	98.4
2′	74.4	74.3	74.0	76.5	73.5	76.1	77.2	75.4	73.8	74.2		73.9	76.4
3′	78.4	78.2[c]	75.0	78.2	76.0	72.4	76.4	78.3	77.6	78.1		74.9	78.1
4′	71.4	71.8[d]	72.0	71.0	70.1	72.1	72.0	72.0	70.9	71.8[j]		71.9	71.1
5′	79.2	78.2[c]	72.1	75.6	73.8	73.2	78.4	78.4	78.6	78.3		71.9	75.6
6′	62.6	69.8	65.6	65.6	63.6	62.8	63.0	63.1	62.2	69.8		65.5	65.1
1″		104.9								104.9			
2″		75.4								75.4			
3″		78.3[c]								78.1			
4″		71.2[d]								71.1[j]			
5″		78.3[c]								78.3			
6″		63.0								63.0			
OCH_2					65.6								
Me					15.1								

[a–k] Assignments are interchangeable.

For **5**, **7** and **15**, there are two columns for each carbon because there are mixtures of α- and β-anomers of the glucose moiety.

of the sugar moiety was in good agreement with that of **3** indicating the structure of **12** to be the β-gentiobiosyl ester of **11**. From the medium of *D. cumminsii* 3-(4-hydroxyphenyl)propionic acid (**13**) was isolated. When 2-phenylpropionic acid (**14**), a branched isomer of **11**, was administered to the suspension culture of *G. echinata*, compound **15** was isolated from the medium. The FABMS spectrum of **15** showed a peak at *m/z* 335 $[M+Na]^+$. The ^1H and ^{13}C NMR spectra of **15** exhibited the signals to be in good agreement with those of the sugar moiety of **5** in addition to the signals due to the 2-phenylpropionyl group. Thus **15** is 6-*O*-(2-phenylpropionyl)-D-glucose.

DISCUSSION

Following the administration of the naturally occurring phenylpropanes, cinnamic acid and *p*-coumaric acid, they were glucosylated and/or hydroxylated by plant cell suspension cultures, but the conversion product of caffeic acid was not observed. Cinnamic acid and *p*-coumaric acid at 100 mg/l show toxicity to the plant suspension cultures and hence the suspension cultures hydroxylated and glycosylated the cinnamic acid and *p*-coumaric acid administered. The cultures temporarily accumulated the products and thereafter they were gradually metabolized. Caffeic acid having two hydroxy group may not be toxic at the concentration of 100 mg/l and hence it may not be necessary for it to be hydroxylated or glycosylated but the caffeic acid adsorbed into the cells may be gradually metabolized. It is reported [8] that the root disks of sweet potato converted *trans*-cinnamic acid supplied externally into the glucosyl ester of cinnamic acid and then that of *p*-coumaric acid. However, in plant cell suspension cultures, the glucosyl ester of cinnamic acid has not been detected. Cell suspension cultures may convert cinnamic acid supplied externally into *p*-coumaric acid and then into its glucosyl ester.

The suspension cultures of *A. japonicum*, *C. arabica* and *G. echinata* converted benzoic acid (**1**, C_6–C_1) into its glucosyl and/or gentiobiosyl esters (**2** and **3**) and those of *A. japonicum* and *C. arabica* also converted 3-phenylpropionic acid (**11**, C_6–C_3) into its gentiobiosyl ester (**12**). On the other hand, with the administration phenylacetic acid (**4**, C_6–C_2), the suspension cultures of *A. japonicum* and *G. echinata* produced the compounds (**5** and **6**) in which **4** was linked to glucose and ethyl glucoside, respectively, at the C-6 position. *Coffea arabica* produced the sucrose ester in which **4** was linked to the C-6 position of the glucose moiety [1]. In the suspension cultures of *A. japonicum* [3] and *G. echinata*, the glycosylation of 2-phenylpropionic acid (**14**), a branched isomer of **11**, was similar to that of **4**. In the suspension culture of *C. arabica* [1, 2], the glycosylation of **14** and its hydroxylated derivatives such as tropic acid and 2-(4-hydroxyphenyl)propionic acid were similar to that of **4**. Therefore the linkage to the C-6 position of the glucose moiety is specific for the C_6–C_2 compound and **14**, and its derivatives, having a branched structure, may be recognized as C_6–C_2 compounds in the suspension cultures of *A. japonicum*, *C. arabica* and *G. echinata*. Although the suspension culture of *C. arabica* mainly attached sucrose

to **4**, **14** and its derivatives, the disaccharide gentiobiose was linked to **1** and **11**. Therefore the formation of the sucrose ester may be peculiar to C_6–C_2 compounds.

The suspension cultures of *A. japonicum*, *C. arabica*, *G. echinata* and *N. tabacum* converted phenylcarboxylic acids into their sugar esters, that is, only glycosylation was observed. However, the suspension culture of *D. cumminsii* converted **4** into phenethyl β-D-glucopyranoside (**8**) and **11**, into 3-(4-hydroxyphenyl)propionic acid (**13**). The former showed the ability to reduce a carboxylic acid into the corresponding primary alcohol and the latter had hydroxylation capability. In the suspension culture of *D. cumminsii*, hydroxylation and reduction of **1**, hydroxylation of **4** and reduction of **11** were not observed and thereby hydroxylation and reduction may be specific for C_6–C_3 and C_6–C_2, respectively. With the administration of **11**, low yields of conversion products and no remains of **11** in suspension cultures, except with *D. cumminsii*, indicated **11** may easily be metabolized by the plant suspension cultures. On the other hand, in the suspension culture of *D. cumminsii*, metabolic activity after hydroxylation may be low and thereby hydroxylated product (**13**) may remain and be excrete into the medium.

EXPERIMENTAL

Mps: uncorr. NMR: 300 or 400 MHz (CD_3OD or $CDCl_3$). FABMS were determined with a JEOL JMS DX-300 instruments equipped with a direct inlet system.

Culture and administration method. The cell culture of *Aconitum japonicum* [3] was initiated in 1978 from root grown in Sado, Japan and subcultured on a Murashige and Skoog's (MS) medium containing 2,4-D 1 ppm and kinetin 0.1 ppm. Thereafter, the callus was transferred in 1980 onto Revised Tobacco (RT) medium [10] containing NAA 1 ppm, kinetin 0.1 ppm and Casamino acid 0.1% and subcultured for 3 weeks. The cell culture of *Dioscoreophyllum cumminsii* [3, 11] was initiated in 1976 from stem on a MS medium containing IAA 1 ppm and kinetin 0.1 ppm, and subcultured on a RT medium containing 2,4-D 1 ppm, kinetin 0.1 ppm and casamino acid 0.1% for 3 weeks. The cell cultures of *Coffea arabica* (seed) [1, 2] and *Nicotiana tabacum* (stem) [3, 12] were initiated in 1982 and 1966, respectively, on MS medium containing 2,4-D 1 ppm and kinetin 0.1 ppm and subcultured for 3 weeks. The cell culture of *Glycyrrhiza echinata* was initiated in 1965 from seedling root on a White medium containing 2,4-D 0.1 ppm and yeast extract 0.1% and subcultured for 3 weeks. Thereafter, in 1976, the callus was treated with MNNG and isolated a strain grown on a MS medium containing IAA 1 ppm and kinetin 0.1 ppm, and subcultured for 3 weeks [13].

These calli were transferred to a liquid medium of the same composition as the subculture, and cultured on a rotary shaker at 145 rpm and 25° in the dark. After 2–3 weeks, each test substrate (25 mg) dissolved in 2 ml 50% EtOH or H_2O, was added to 250 ml of each suspension culture and cultured for 1 or 3 days. The test substrates were benzoic acid, *trans*-cinnamic acid, *trans*-*p*-coumaric acid, *trans*-caffeic acid, 3-phenylpropionic acid, purchased from Wako Pure Chemical (Osaka), phenylacetic acid (colourless plates, mp 76–77°, bp 265–266°) and 2-phenylpropionic acid (colourless liquid, bp 264–265°) supplied by Nissan Chemical Industries Ltd.

Isolation of the conversion products from benzoic acid. The suspension culture of *A. japonicum* (0.5 l) previously administered benzoic acid was separated into the medium and the cells by Nylon cloth, and the cells were homogenized with MeOH and filtered. The filtrate was concd and applied on a Diaion HP-20 column and washed with H_2O followed by elution with MeOH. From the eluate, compounds **2** (10 mg) and **3** (21.1 mg) were isolated by silica gel (Wako gel C-200) CC (eluent, $CHCl_3$–MeOH–H_2O, 20:6:1) and HPLC using Senshu Pak ODS-4301-N column and MeOH–H_2O (3:7 for **2** and 1:4 for **3**) as the eluents.

From the suspension cultures of *C. arabica* (0.5 l), *D. cumminsii* (2 l), *G. echinata* (1 l) and *N. tabacum* (1 l), compounds **2** (11.1, 12.1, 16.8, 5.2 mg, respectively) and **3** (5.9, 6.0, 0, 11.1 mg, respectively) were isolated by the similar method to that used with *A. japonicum*. The eluent of the silica gel column (Wako gel C-200) was $CHCl_3$–MeOH–H_2O (6:4:1) and the eluents for HPLC (column, Senshu Pak ODS-4301-N) were MeOH–H_2O (3:7) for **2** and MeOH–H_2O (1:4) for **3**.

Isolation of compound **5**. From the *n*-BuOH extract of the medium of *A. japonicum* previously administered phenylacetic acid (total 350 mg), compound **5** (10.4 mg) was isolated by silica gel (Kieselgel 60) CC (eluent, $CHCl_3$–MeOH, 8:1) and HPLC using a Unisil Q C18 column and MeOH–H_2O (3:7) as the eluent.

Isolation of compound **6**. From the EtOAc extract of the medium of *G. echinata* previously administered phenylacetic acid (total 400 mg), compound **6** (46 mg) was isolated by HPLC using Unisil Q C18 column and MeOH–H_2O (1:1 and 2:3) as the eluent.

Isolation of compound **7** *and* **8**. The MeOH extract of the cultured cells of *D. cumminsii* previously administered phenylacetic acid (total 400 mg) was suspended in H_2O and extracted with *n*-BuOH saturated with H_2O. From the *n*-BuOH extract, compounds **7** (2 mg) and **8** (4.6 mg) were isolated by silica gel (Wako gel C-200 and Kieselgel 60) CC ($CHCl_3$–MeOH–H_2O, 7:3:1, lower layer and EtOAc–MeOH, 12:1) and HPLC using Unisil Q C18 column and MeOH–H_2O (3:7 and 1:3) and MeOH–THF–H_2O (3:2:20) as the eluents.

Isolation of compound **10**. The MeOH extract of the cultured cells of *N. tabacum* previously administered *trans*-cinnamic acid (total 200 mg) was partitioned between EtOAc and H_2O. From the aq. layer, compound **10** (22.0 mg) was isolated by silica gel (Wako gel C-200) CC (eluent, $CHCl_3$–MeOH–H_2O, 6:4:1) and HPLC using Senshu Pak ODS-4301-N column and MeOH–H_2O (3:7) as the eluent, and crystallized from MeOH–H_2O.

Isolation of compound **12**. The MeOH extracts of the cultured cells of *A. japonicum* (2 l) and *C. arabica* (2 l) previously administered 3-phenylpropionic acid were suspended in H_2O. The suspension was applied on a Diaion HP-20 column and washed with H_2O followed by elution with MeOH. From the MeOH eluates, compound **12** (*A. japonicum*, 5.1 mg; *C. arabica*, 21.4 mg) was isolated by silica gel (Wako gel C-200) CC (eluent, $CHCl_3$–MeOH, 4:1) and HPLC using Senshu Pak ODS-4301-N column and MeOH–H_2O (1:3 or 1:4) as the eluent.

Isolation of compound **13**. From the EtOAc extract of the medium of *D. cumminsii* suspension culture administered 3-phenylpropionic acid (total 300 mg), compound **13** (16.1 mg) was isolated by silica gel (Wako gel C-200) CC (eluent, $CHCl_3$–MeOH, 20:1) and HPLC using Senshu Pak ODS-4301-N column and MeOH–H_2O (3:7) as the eluent.

Isolation of compound **15**. The medium of *G. echinata* previously administered 2-phenylpropionic acid (total 250 mg) was partitioned between EtOAc and H_2O. The aq. layer was extracted with *n*-BuOH saturated with H_2O. From the *n*-BuOH extract compound **15** (9.6 mg) was isolated by HPLC using Unisil Q C18 column and MeOH–H_2O (2:3) as the eluent.

Identification of p-*coumaric acid, its glucosyl ester* (**10**) *and caffeic acid.* The suspension cultures previously administered

cinnamic acid, *p*-coumaric acid or caffeic acid were separated into the media and the cells by Nylon cloth, and the latter were homogenized with MeOH. The homogenates were filtered and evapd. The media and the MeOH extracts were applied on a Diaion HP-20 column and washed with H_2O followed by elution with MeOH. Cinnamic acid, *p*-coumaric acid, caffeic acid and/or the glucosyl ester of *p*-coumaric acid (**10**) containing the MeOH eluates were identified with authentic cinnamic acid, *p*-coumaric acid, caffeic acid and **10** isolated from the suspension culture of *N. tabacum* administered cinnamic acid by TLC using C_6H_6–dioxane–HOAc (18:5:1) and $CHCl_3$–MeOH–H_2O (6:4:1) as the solvents, and detected by 10% $FeCl_3$, UV absorption, 10% $FeCl_3$–5% $K_3Fe(CN)_6$ and 10% H_2SO_4 (heat).

Benzoyl 6-O-*β*-D-*glucopyranosyl*-*β*-D-*glucopyranoside* (**3**). Amorphous solid: $[\alpha]_D^{20}$ −27.8° (MeOH; *c* 1.10); IR ν_{max}^{KBr} cm^{-1}: 3405, 1725; ^1H NMR (CD_3OD): δ5.80 (1H, *d*, *J* = 7.5 Hz, H-1′), 4.28 (1H, *dd*, *J* = 11.5, 2 Hz) and 3.89 (1H, *dd*, *J* = 11.5, 5 Hz, H-6′), 4.43 (1H, *d*, *J* = 7.5 Hz, H-1″), 3.30 (1H, *dd*, *J* = 9, 7.5 Hz, H-2″), 3.74 (1H, *ddd*, *J* = 10, 5.5, 2 Hz, H-5″), 3.92 (1H, *dd*, *J* = 12, 2 Hz) and 3.73 (1H, *dd*, *J* = 12, 5.5 Hz, H-6″), 8.17–8.22 (2H, *m*, H-3 and H-7), 7.56–7.63 (2H, *m*, H-4 and H-6), 7.70–7.77 (1H, *m*, H-5), 3.57–3.66 (3H, *m*, H-2′, H-3′ and H-5′), 3.34–3.47 (3H, *m*, H-4′, H-3″ and H-4″); ^{13}C NMR (CD_3OD): see Table 1; FABMS *m/z*: 447 $[MH]^+$.

6-O-*Phenylacetyl*-D-*glucose* (**5**). Amorphous solid: $[\alpha]_D^{19}$ +32.6° (MeOH; *c* 0.43); IR ν_{max}^{KBr} cm^{-1}: 3420, 1725; ^1H NMR (CD_3OD): δ3.75 (1.2H, *s* and 3.76, 0.8H, *s*, H-2), 5.15 (0.6H, *d*, *J* = 3.8 Hz) and 4.57 (0.4H, *d*, *J* = 8 Hz, H-1′), 3.22 (0.4H, *dd*, *J* = 9, 8 Hz, H-2′), 3.75 (0.6H, *t*, *J* = 9 Hz, H-3′), 3.36 (0.6H, *dd*, *J* = 10, 9 Hz) and 3.37 (0.4H, *t*, *J* = 9.5 Hz, H-4′), 4.04 (0.6H, *ddd*, *J* = 10, 5, 2 Hz) and 3.56 (0.4H, *ddd*, *J* = 9.5, 6, 2 Hz, H-5′), 4.49 (0.6H, *dd*, *J* = 11.5, 2 Hz), 4.52 (0.4H, *dd*, *J* = 11.5, 2 Hz), 4.31 (0.6H, *dd*, *J* = 11.5, 5 Hz) and 4.29 (0.4H, *dd*, *J* = 11.5, 6 Hz, H-6′), 7.31–7.42 (5H, *m*, H-4, H-5, H-6, H-7 and H-8), 3.39–3.45 (1H, *m*, H-2′ and H-3′); ^{13}C NMR (CD_3OD): see Table 1; FABMS *m/z*: 321 $[M+Na]^+$.

Ethyl 6-O-*phenylacetyl*-*β*-D-*glucopyranoside* (**6**). Amorphous solid: $[\alpha]_D^{18}$ −19.5° (MeOH; *c* 0.88); IR ν_{max}^{KBr} cm^{-1}: 3500, 1730; ^1H NMR ($CDCl_3$): δ3.77 (2H, *s*, H-2), 4.23 (1H, *d*, *J* = 8 Hz, H-1′), 3.33 (1H, *dd*, *J* = 9, 8 Hz, H-2′), 3.33 (1H, *dd*, *J* = 9.5, 8.5 Hz, H-4′), 3.44 (1H, *ddd*, *J* = 9.5, 5, 2.5 Hz, H-5′), 4.41 (1H, *dd*, *J* = 12, 5 Hz) and 4.35 (1H, *dd*, *J* = 12, 2.5 Hz, H-6′). 1.23 (3H, *t*, *J* = 7 Hz, Me), 7.23–7.35 (5H, *m*, H-4, H-5, H-6, H-7 and H-8), 3.83–3.91 (1H, *m*, OCH_2), 3.49–3.57 (2H, *m*, H-3′ and OCH_2); ^{13}C NMR ($CDCl_3$): see Table 1; FABMS *m/z*: 349 $[M+Na]^+$.

2-O-*Phenylacetyl*-D-*glucose* (**7**). Amorphous solid: $[\alpha]_D^{21}$ −2.5° (MeOH; *c* 0.55); IR ν_{max}^{KBr} cm^{-1}: 3415, 1720; ^1H NMR (CD_3OD): δ3.71, 2H (*s*, H-2), 5.23 (0.6H, *d*, *J* = 3.8 Hz) and 4.60 (0.4H, *d*, *J* = 8 Hz, H-1′), 4.57 (0.6H, *dd*, *J* = 10, 3.8 Hz) and 4.68 (0.4H, *dd*, *J* = 9.5, 8 Hz, H-2′), 3.90 (0.6H, *dd*, *J* = 10, 9 Hz) and 3.50 (0.4H, *dd*, *J* = 9.5, 8.5 Hz, H-3′), 3.38 (0.6H, *dd*, *J* = 9.5, 9 Hz) and 3.35 (0.4H, *t*, *J* = 8.5 Hz, H-4′), 7.27–7.32 (4H, *m*, H-4, H-5, H-7 and H-8), 7.21–7.25 (1H, *m*, H-6); ^{13}C NMR (CD_3OD): see Table 1; FABMS *m/z*: 321 $[M+Na]^+$.

3-*Phenylpropionyl* 6-O-*β*-D-*glucopyranosyl*-*β*-D-*glucopyranoside* (**12**). Amorphous solid: $[\alpha]_D^{19}$ −12.6° (MeOH; *c* 1.06); IR ν_{max}^{KBr} cm^{-1}: 3400, 1745; ^1H NMR (CD_3OD): δ5.56 (1H, *d*, *J* = 8 Hz, H-1′), 3.30 (1H, *dd*, *J* = 9, 8 Hz, H-2′), 3.63 (1H, *ddd*, *J* = 9.5, 5.2 Hz, H-5′), 4.24 (1H, *dd*, *J* = 11.5, 2 Hz) and 3.86 (1H, *dd*, *J* = 11.5, 5 Hz, H-6′), 4.42 (1H, *d*, *J* = 7.6 Hz, H-1″), 3.97 (1H, *dd*, *J* = 12, 1.8 Hz) and 3.75 (1H, *dd*, *J* = 12, 5 Hz, H-6″), 3.04 (2H, *br t*, *J* = 7.5 Hz, H-2), 2.77–2.84 (2H, *m*, H-3), 7.24–7.39 (5H, *m*, H-5, H-6, H-7, H-8 and H-9), 3.34–3.56 (6H, *m*, H-3′, H-4′, H-2″, H-3″, H-4″ and H-5″); ^{13}C NMR (CD_3OD): see Table 1; FABMS *m/z*: 497 $[M+Na]^+$.

(2RS)-6-O-(2-*phenylpropionyl*)-D-*Glucose* (**15**). Amorphous solid: $[\alpha]_D^{21}$ +34.3° (MeOH; *c* 0.49); IR ν_{max}^{KBr} cm^{-1}: 3430, 1725; ^1H NMR (CD_3OD): δ3.770 and 3.765 (each 0.5H, *q*, *J* = 7 Hz, H-2), 1.456 and 1.455 (each 1.5H, *d*, *J* = 7 Hz, H-3), 4.98 (0.6H, *d*, *J* = 3.8 Hz) and 4.42 (0.4H, *d*, *J* = 8 Hz, H-1′), 3.19 (0.6H, *dd*, *J* = 9.5, 3.8 Hz) and 3.08 (0.4H, *dd*, *J* = 9, 8 Hz, H-2′), 3.61 (0.6H, *dd*, *J* = 9.5, 9 Hz) and 3.30 (0.4H, *t*, *J* = 9 Hz, H-3′), 3.19 (0.6H, *dd*, *J* = 10, 9 Hz) and 3.22 (0.4H, *dd*, *J* = 9.5, 9 Hz, H-4′), 3.87 (0.6H, *ddd*, *J* = 10, 5, 2.2 Hz) and 3.40 (0.4H, *ddd*, *J* = 9.5, 6, 2 Hz, H-5′), 4.38 (0.6H, *dd*, *J* = 12, 2.2 Hz), 4.37 (0.4H, *dd*, *J* = 12, 2 Hz), 4.19 (0.6H, *dd*, *J* = 12, 5 Hz) and 4.20 (0.4H, *dd*, *J* = 12, 6 Hz, H-6′), 7.19–7.24 and 7.27–7.32 (total 5H, *m*, H-5, H-6, H-7, H-8 and H-9); ^{13}C NMR (CD_3OD): see Table 1; FABMS *m/z*: 335 $[M+Na]^+$.

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