Phytochemistry, Vol. 27, No. 3, pp. 803–807, 1988.
Printed in Great Britain.

0031-9422/88 $3.00+0.00

BIOTRANSFORMATION OF PHENYLACETIC ACID AND 2-PHENYLPROPIONIC ACID IN SUSPENSION CULTURE OF *COFFEA ARABICA**

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(*Received* 11 *August* 1987)

Key Word Index—*Coffea arabica*; Rubiaceae; cell suspension culture; biotransformation; glycosylation; sucrose ester; phenylacetic acid; 2-(*R*, *S*)-phenylpropionic acid.

Abstract—Suspension cultures of *Coffea arabica* are able to convert phenylacetic acid and 2-(*R*, *S*)-phenylpropionic acid and its ethyl ester into their sucrose esters, β-D-fructofuranosyl 6-*O*-phenylacetyl-α-D-glucopyranoside and β-D-fructofuranosyl 6-*O*-[2-(*R*, *S*)-phenylpropionyl]-α-D-glucopyranoside, which are accumulated in the cells. The diastereomeric mixture of the sucrose ester of 2-(*R*, *S*)-phenylpropionic acid (*R*:*S*::1:1) was resolved by HPLC.

INTRODUCTION

Numerous phenylpropanoids and closely related compounds of natural or synthetic origin are available for human use. To obtain compounds which are pharmacologically more active and which are difficult to synthesize chemically, we have investigated the ability of plant cell cultures to biotransform phenylpropanoids and their analogues such as phenylacetic acid and 2-(*R*, *S*)-phenylpropionic acid. In a previous paper [1], we reported that suspension cultures of *Nicotiana tabacum*, *Dioscoreophyllum cumminsii* and *Aconitum japonicum* are able to convert 2-phenylpropionic acid and its ethyl ester into their glucosyl and gentiobiosyl esters. The present paper reports on the biotransformation of phenylacetic acid, 2-phenylpropionic acid and ethyl 2-phenylpropionate to their sucrose esters by suspension culture of *Coffea arabica*.

RESULTS AND DISCUSSION

Compounds **1** and **2** were isolated from cultured cells of *C. arabica* previously administered ethyl 2-phenylpropionate. On acid hydrolysis with 0.05 M H_2SO_4 at 70° each compounds gave 2-phenylpropionic acid, fructose, glucose and compound **4**. Acid hydrolysis of **4** with 0.5 M H_2SO_4 at 70° gave 2-phenylpropionic acid and glucose. The FD MS spectra of **1** and **2** showed a peak at *m/z* 475 $[MH]^+$ suggesting that both compounds contained one molecule each of 2-phenylpropionic acid, glucose and fructose. The assignment of each signal in the NMR spectra of **1** and **2** was performed by ^1H–^1H and ^1H–^{13}C-2D NMR (Tables 1 and 2). The chemical shifts and coupling constants of the sugar moiety of **1** were in good agreement with those of sucrose [2–4]. However, in the ^1H NMR spectrum, an acylation shift (δ0.6) of H-6 of the glucose moiety suggested the 2-phenylpropionyl group was attached to C-6 of the glucose residue of the sucrose moiety.

The ^{13}C NMR spectrum of **2** was very similar to that of **1**. Slight differences in the chemical shifts suggested that the configuration at C-2 of the 2-phenylpropionyl group was different in each compound. The CD spectrum of **1** showed a negative maximum at 221 (Δε −5.33) and that of **2**, a positive maximum at 221 (Δε 3.45). The CD spectrum of **2** exhibited the same Cotton effect as (2*S*)-phenylpropionic acid and that of **1**, the reverse, indicating the configuration of **1** to be *R* and that of **2**, S. From these results **1** was assigned the structure of β-D-fructofuranosyl 6-*O*-[(2*R*)-phenylpropionyl]-α-D-glucopyranoside and **2**, β-D-fructofuranosyl 6-*O*-[(2*S*)-phenylpropionyl]-α-D-glucopyranoside.

Compound **3** was isolated from cultured cells administered phenylacetic acid. Its FDMS spectrum showed a peak (*m/z* 461 $[MH]^+$), which was 14 mass units lower than that of **1**. In the ^1H and ^{13}C NMR spectra, the chemical shifts of the sugar moiety of **3** were similar to those of **1** and **2**, thus establishing that **3** was β-D-fructofranosyl 6-*O*-phenylacetyl-α-D-glucopyranoside.

To investigate the ability of *C. arabica* to biotransform each substrate, time course experiments were carried out. Suspension cultures of *C. arabica*, precultured for two weeks, were administered 2-phenylpropionic acid, ethyl 2-phenylpropionate or phenylacetic acid and harvested at days one to seven. The suspension culture converted 2-phenylpropionic acid and its ethyl ester into the conversion products **1**, **2**, **5** and **6**, which were accumulated in the cells and could not be detected in the medium (Fig. 1). The structures of **5** and **6** had been established as 2-(*R*, *S*)-phenylpropionyl β-D-glucopyranoside and 2-(*R*, *S*)-phenylpropionyl 6-*O*-β-D-glucopyranosyl-β-D-glucopyranoside, respectively, in a previous study [1]. When the suspension culture was administered 2-phenylpropionic acid, its conversion into **1**, **2**, **5** and **6** was observed one day after administration. The conversion ratio into **1** and **2** increased and reached a maximum (about 25%) at four days after which it decreased gradually. The conversion ratio into **5** was about 9% after day one and then

*Part 51 in the series 'Studies on Plant Tissue Culture'. For Part 50 see Ref. [1].

Table 1. ^{1}H NMR data for compounds **1–3** (400 MHz, CD_3OD)

H		1	2	3
2	1H, *q*	3.81 (7)*	3.81 (7)	3.69 (2H, *s*)
3	3H, *d*	1.46 (7)	1.47 (7)	—
5, 6, 8, 9	4H, *m*	7.28–7.32	7.29–7.33	—
4, 5, 7, 8	4H, *m*	—	—	7.27–7.31
6	1H, *m*	—	—	7.22–7.26
7	1H, *m*	7.20–7.26	7.20–7.25	—
Glc-1	1H, *d*	5.26 (3.8)	5.33 (3.8)	5.35 (3.8)
2	1H, *dd*	3.22 (10, 3.8)	3.33 (9.5, 3.8)	3.36 (10, 3.8)
3	1H, *dd*	3.64 (10, 9)	3.67 (9.5, 9)	3.80 (10, 9)
4	1H, *dd*	3.20 (10, 9)	3.22 (9.5, 9)	3.25 (9.5, 9)
5	1H, *ddd*	3.93 (10, 4.3, 2)	3.99 (9.5, 4.4, 2)	4.02 (9.5, 5, 2.2)
6a	1H, *dd*	4.39 (12, 2)	4.48 (12, 2)	4.44 (12, 2.2)
6b	1H, *dd*	4.21 (12, 4.3)	4.11 (12, 4.4)	4.18 (12, 5)
Fru-1a	1H, *d*	3.55 (12)	3.57 (12)	3.57 (12)
1b	1H, *d*	3.60 (12)	3.62 (12)	3.62 (12)
3	1H, *d*	4.08 (8)	4.09 (8)	4.10 (8)
4	1H, *t*	3.97 (8)	3.98 (8)	4.00 (8)
5, 6	3H, *m*	3.72–3.80	3.70–3.80	3.66–3.82

*J in Hz shown in parentheses.

Table 2. ^{13}C NMR data for compounds **1–3** (400 MHz, CD_3OD)

C	1	2	C	3
1	176.5	176.6	1	173.8
2	46.8	46.9	2	42.1
3	19.4	19.5		
4	142.4	142.3	3	135.9
5, 9	129.9*	129.9†	4, 8	130.8‡
6, 8	129.0*	128.9†	5, 7	129.8‡
7	128.5	128.5	6	128.3
Glc-1	93.7	93.8	Glc-1	93.7
2	73.3	73.4	2	73.4
3	74.6	74.7	3	74.7
4	71.6	71.7	4	71.9
5	72.5	72.4	5	72.3
6	64.8	65.0	6	65.4
Fru-1	64.4	64.4	Fru-1	64.4
2	105.5	105.6	2	105.6
3	79.6	79.5	3	79.5
4	76.2	76.2	4	76.2
5	84.2	84.2	5	84.2
6	64.1	64.1	6	64.2

*†‡ Assignments are interchangeable.

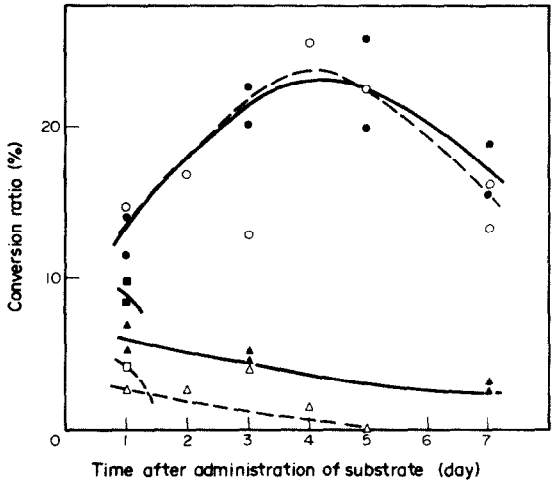

Fig. 1. Time course of formation of conversion products from 2-phenylpropionic acid (PPA) or its ethyl ester (PPA-E) in the cells of *C. arabica*: ○——○, **1**+**2** from PPA; ●——●, **1**+**2** from PPA-E; □ - - - □, **5** from PPA; ■——■, **5** from PPA-E; △ - - - △, **6** from PPA; ▲——▲, **6** from PPA-E.

decreased rapidly: **5** was not detected at day two. This result suggested that **5** was converted into **1** and **2** by the action of a glycosyltransferase. The conversion ratio into **6** was about 3% at day one after which **6** decreased gradually with time. The administration of ethyl 2-phenylpropionate gave a similar result to 2-phenylpropionic acid (Fig. 1). The suspension culture of *C. arabica* showed no difference in the utilization of the free acid and ester forms, unlike suspension cultures of *N. tabacum*, *D. cummunsii* and *A. japonicum*. It was unable to stereoselectively form the sucrose esters of (2*R*)-phenylpropionic acid, (2*S*)-phenylpropionic acid or their ethyl esters. However, the diastereoisomers of the sucrose esters of 2-(*R*,*S*)-phenylpropionic acid could be resolved by HPLC.

On administration of phenylacetic acid (Fig. 2), the formation of **3** was observed at day one, and the maximum conversion ratio was observed at days two. It was formed more rapidly than when 2-phenylpropionic acid or its ethyl ester was administered, but the conversion ratio of 15% was lower than that from 2-phenylpropionic acid or

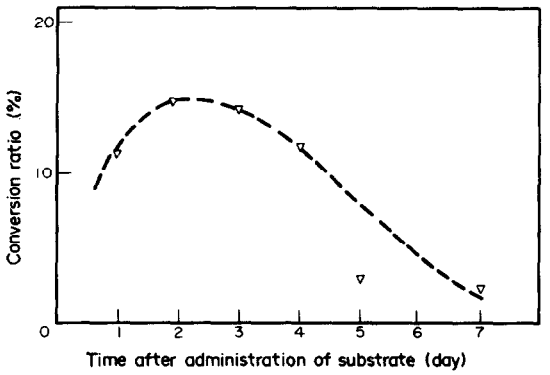

Fig. 2 Time course of conversion of phenylacetic acid into **3** in the cells of *C. arabica*.

its ethyl ester. After maximum conversion, **3** decreased more rapidly than **1** and **2**. Glucosyl and gentiobiosyl esters of phenylacetic acid corresponding to **5** and **6** were not detected. Phenylacetic acid has a more similar structure to phenylpropane than 2-phenylpropionic acid, therefore, it may be a better substrate than 2-phenylpropionic acid and its products may be metabolized more easily.

In glycosylation reactions of plant cell cultures, it is well known that glucose [5–15] or its oligomers such as gentiobiose [6, 10, 11] attach to administered substrates. However, it is a rare for sugars such as sucrose to be attached to administered substrates. Recently, sucrose esters of phenylpropanes have been isolated from several plants [3, 16–19]. However, this is the first report of the formation of sucrose esters as biotransformation products.

The formation of sucrose esters by the suspension culture of *C. arabica* took place in a medium which contained sucrose as carbon source. It will be of interest, therefore, to see if the nature of the sugar residue attached to the substrate can be altered by replacement of the sucrose in the growth medium with other sugars.

EXPERIMENTAL

NMR spectra were determined in CD_3OD at 400 MHz. FDMS was taken with a JEOL JMS D-300 and DX-300 instruments equipped with a direct inlet system. GC was conducted on a Shimadzu GC-9A GC provided with a hydrogen FID. A glass column (2 m × 0.3 mm) packed with 3% ECNSS-M on Gas Chrom Q(100–120 mesh) was operated at 195° at a gas flow rate of 50 ml of N_2 per min.

Culture methods. The cell culture of *Coffea arabica* was initiated in 1982 from seed, and subcultured on Murashige and Skoog's agar medium containing 2,4-D(1 ppm) and kinetin (0.1 ppm) at 25° in the dark for 3 weeks. Callus, which had been cultured on agar medium for 3 weeks, was transferred to the liquid medium of the same composition and cultured on a rotary shaker at 145 rpm and 25° in the dark. After 2 weeks, 25 mg of the test substrate dissolved in 2 ml 50% EtOH was added to 250 ml suspension culture which was then cultured for a period of from 1 to 7 days. The substrates used in this experiment were phenylacetic acid (colourless plates, mp 76–77°, bp 265–266°), 2-(*R, S*)-phenylpropionic acid (colourless liquid, bp 264–265°) and ethyl 2-(*R, S*)-phenylpropionate(colourless liquid, bp 230°) (Nissan Chemical Industries Ltd).

Isolation of compounds **1** *and* **2**. The suspension culture administered ethyl 2-phenylpropionate was separated into medium and cells by filtration through Nylon cloth. The cells were homogenized in MeOH, the homogenate filtered and the residue extracted with MeOH under reflux for 3 hr. The MeOH extracts were combined, concd, suspended in H_2O, and partitioned between *n*-BuOH and H_2O. The *n*-BuOH fraction was chromatographed on a column of silica gel (WAKO gel C-200) using CH_2Cl_2–MeOH–H_2O (7:3:1, lower layer) as the eluent. The fraction containing the products was rechromatographed on a column of silica gel using $CHCl_3$–MeOH (3:1) as the eluent. Compounds **1** and **2** were isolated from the eluate by HPLC using a Unisil Q C18 column with MeOH–H_2O (3:7) as the eluent.

Isolation of compound **3**. An *n*-BuOH extract obtained by the same method as that just described from a suspension culture administered phenylacetic acid, was chromatographed on a column of silica gel using $CHCl_3$–MeOH (3:1) as the eluent. Compound **3** was isolated from the eluate by HPLC using a Unisil Q C18 column and MeOH–H_2O (3:7) as the eluent.

Quantitative analysis of conversion products. The media and MeOH extracts of the cells from 250 ml of suspension culture which had been incubated for 1,2,3,4,5 or 7 days with the test substrate were extracted (× 2) with *n*-BuOH saturated with H_2O. The amount of each of the conversion products present in the *n*-BuOH extracts was determined by HPLC: Unisil Q C18 column (300 × 7.6 mm), MeOH–H_2O–AcOH (19:30:1), detection by differential refractometer and UV (254 nm) absorption. R_t(min) of **1**, **2**, **3**, **5**, **6**, phenylacetic acid and 2-phenylpropionic acid were 13.3, 14.2, 8.4, 15.1, 10.8, 19.8 and 39.2, respectively. Ethyl 2-phenylpropionate was not detected in the eluent.

Acid hydrolysis of the conversion products. The conversion products were hydrolysed with 0.05 M H_2SO_4 in a sealed tube attached to a vacuum system at 70° for 10 hr. The hydrolysates were applied to a column of Diaion HP-20 and eluted with H_2O and MeOH. The water eluates were neutralized by passage through a column of amberlite IRA 45(OH^-). After concn of the eluates, the sugars in the eluates were identified with authentic glucose and fructose by TLC (R_f 0.30 and 0.40 Me_2CO–H_2O–$CHCl_3$–MeOH, 15:1:2:2) and by GC as their alditol acetates [1]. The MeOH eluates were taken to dryness and further hydrolysed with 0.5 M H_2SO_4 in a sealed tube to a vacuum system at 70° for 5 hr. The hydrolysates were applied to a column of Diaion HP-20 and eluted with MeOH following elution of sugars with H_2O. The 2-phenylpropionic acid or phenylacetic acid in the eluate was identified by TLC (R_f 0.56 and 0.46, C_6H_6–dioxane–AcOH, 18:5:1) with authentic samples. The water eluates were neutralized by passage through a column of Amberlite IRA 45(OH^-). After elution with H_2O, the sugar in the eluate were identified as glucose by TLC (R_f 0.30, Me_2CO–H_2O–$CHCl_3$–MeOH, 15:1:2:2), and by GC as its alditol acetate.

β-D-Fructofuranosyl 6-O-[(2R-*phenylpropionyl*]-α-D-*glucopyranoside* (**1**). Amorphous solid: $[\alpha]_D^{27}$ +24.8° (EtOH; *c* 1.24), UV λ_{max}^{EtOH} nm (log ε): 204 (3.91), 251 (2.18), 258 (2.25), 264 (2.13); IR ν_{max}^{KBr} cm^{-1}: 3400, 1730; ^1H NMR (CD_3OD): see Table 1; ^{13}C NMR (CD_3OD): see Table 2. FD MS *m/z*: 475 $[MH]^+$; CD (EtOH; *c* 2.62 × 10^{-4}) $\Delta\varepsilon^{23}$: −5.33 (221) (neg. max).

β-D-Fructofuranosyl 6-O-[(2S-*phenylpropionyl*]-α-D-*glucopyranoside* (**2**). Amorphous solid: $[\alpha]_D^{27}$ +53.0° (EtOH; *c* 1.35), UV λ_{max}^{EtOH} nm (log ε): 204 (3.90), 251 (1.89), 257 (2.05), 263 (1.85); IR ν_{max}^{KBr} cm^{-1}: 3400, 1730; ^1H NMR (CD_3OD): see Table 1; ^{13}C NMR (CD_3OD): see Table 2; FD MS *m/z*: 475 $[MH]^+$; CD (EtOH; *c* 2.85 × 10^{-4}) $\Delta\varepsilon^{23}$: 3.45 (221) (pos. max).

β-D-Fructofuranosyl 6-O-*phenylacetyl*-α-D-*glucopyranoside* (**3**). Amorphous solid: $[\alpha]_D^{27}$ +46.9° (EtOH; *c* 1.21); UV λ_{max}^{EtOH} nm

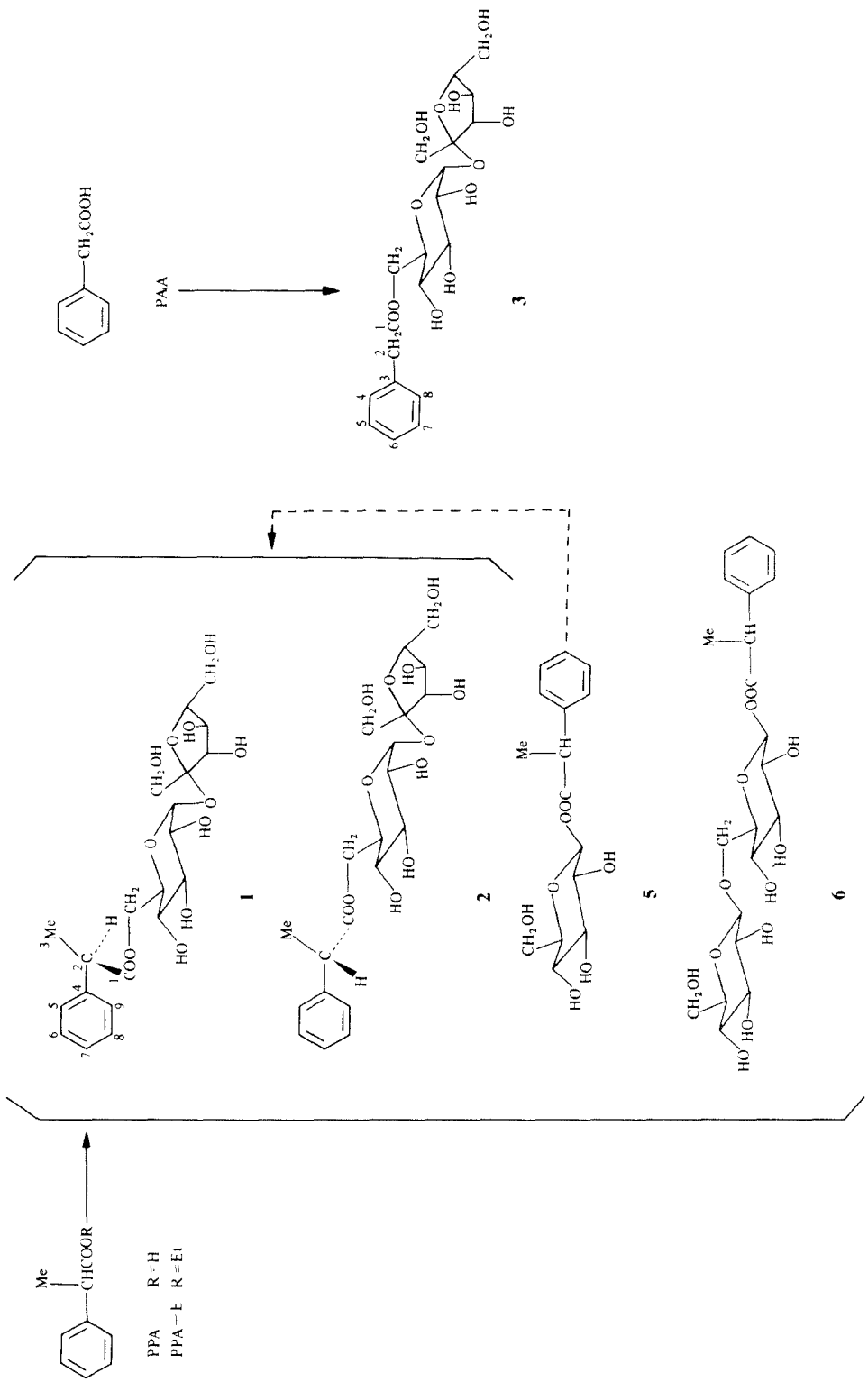

Fig. 3. Possible scheme for biotranformation of phenylacetic acid (PAA), 2-phenylpropionic acid (PPA) and ethyl 2-phenylpropionate (PPA-E) by cell culture of *C. arabica*.

(log ε): 205 (3.74), 251 (2.11), 257 (2.21), 263 (2.06); IR ν_{max}^{KBr} cm^{-1}: 3390, 1725; ^1H NMR (CD_3OD): see Table 1; ^{13}C NMR (CD_3OD): see Table 2; FDMS *m/z*: 461 $[MH]^+$.

Acknowledgements—We thank Nissan Chemical Industries Ltd. for kindly providing the phenylacetic acid, 2-(*R, S*)-phenylpropionic acid and its ethyl ester and the members of the Analytical Center of this University for NMR and MS measurements.

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