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BIOTRANSFORMATION OF 2-PHENYLPROPIONIC ACID IN ROOT CULTURE OF *PANAX GINSENG**

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Key Word Index—*Panax ginseng*; Araliaceae; plant tissue culture; root culture; biotransformation; glucosyl ester; primeverosyl ester; inositol ester; (*RS*)-2-phenylpropionic acid.

Abstract—Root cultures of *Panax ginseng* are able to convert (*RS*)-2-phenylpropionic acid into (*RS*)-2-phenylpropionyl β-D-glucopyranoside, (2*RS*)-2-*O*-(2-phenylpropionyl)-D-glucose, (2*S*)-2-phenylpropionyl 6-*O*-β-D-xylopyranosyl-β-D-glucopyranoside and a *myo*-inositol ester of (*R*)-2-phenylpropionic acid. The primeverosyl ester and *myo*-inositol ester were formed stereoselectively at C-2 from the racemic mixture. The conversion into glucosyl esters was very high at *ca* 90% and the total conversion reached 100% at day three. About a half of the products were excreted into the medium. Compared with the root culture, the callus showed lower glycosylation ability.

INTRODUCTION

We have investigated the ability of plant cell cultures to biotransform phenylpropanoids and their analogues. In previous papers [1, 2], we reported that suspension cultures of *Nicotiana tabacum*, *Dioscoreophyllum cumminsii* and *Aconitum japonicum* are able to convert 2-phenylpropionic acid and its ethyl ester to their glucosyl and gentiobiosyl esters, and that *Coffea arabica*, converts these and phenylacetic acid into their sucrose esters. In our previous papers [3–5], we reported that ginseng (*Panax ginseng* C. A. Meyer) callus produces almost the same pharmacologically active saponins, ginsenosides, as those of the original plants. (Cultured root [5] redifferentiated from the callus produced the highest content of the saponins. Therefore, we investigated the glycosylation of phenylpropanoids by the ginseng cultured root, which must have the highest glycosylation activity. The present paper reports on the biotransformation of 2-phenylpropionic acid in root culture of *Panax ginseng*.

RESULT AND DISCUSSION

Compounds **1**–**4** were isolated from root culture of *Panax ginseng* previously administered 2-phenylpropionic acid. The ^{1}H and ^{13}C NMR spectra of **1** agreed with that of (2*RS*)-2-phenylpropionyl β-D-glucopyranoside [1] (Tables 1 and 2). The NMR spectra of **2** exhibited signals showing the presence of α- and β-anomers of glucose moiety in addition to those due to (*RS*)-2-phenylpropionyl group. The ratio of α- and β-anomers was judged to be *ca* 2:3 from the integrated intensity of the respective anomeric protons. In the ^{1}H NMR spectrum of **2**, the H-2 signals of glucose moiety shifted downfield (δ 4.69, 0.61 H, *dd*, J = 9.5, 8 Hz, δ 4.58, 0.23 H, *dd*, J = 10, 3.8 Hz and δ 4.54, 0.16 H, *dd*, J = 10, 3.8 Hz) as compared with that of **1**, indicating the (*RS*)-2-phenylpropionyl group to be attached to the C-2 position of the glucose moiety.

The FABMS spectrum of **3** showed a peak at *m/z* 467 $[M+Na]^+$ suggesting the composition of **3** to be one molecule each of 2-phenylpropionic acid, hexose and pentose. The assignment of each signal in the NMR spectra of **3** was performed by ^{1}H–^{1}H and ^{1}H–^{13}C 2D NMR. The chemical shifts and coupling constants of the sugar moiety of **3** were in good agreement with those of β-D-glucopyranose and β-D-xylopyranose [6, 7]. However, the glycosylation shifts (δ + 7.0 and δ + 8.0, respectively) of the C-6 of glucose moiety and the anomeric carbon of xylose moiety, respectively, suggested that the sugar moiety was primeverose with the xylose attached to the C-6 of glucose and an acylation shift (δ + 1.08) of the anomeric proton of glucose moiety suggested the 2-phenylpropionyl group was attached at C-1 of the glucose. The CD spectrum of **3** showed a positive maximum at 221 (Δε: + 4.43) and exhibited the same Cotton effect as (2*S*)-2-phenylpropionyl β-D-glucopyranoside, produced from (*S*)-2-phenylpropionic acid by biotransformation using the root culture of *P. ginseng*, indicating the configuration at C-2 of the 2-phenylpropionyl group of **3** to be *S*. From these results **3** was assigned the structure of (2*S*)-2-phenylpropionyl 6-*O*-β-D-xylopyranosyl-β-D-glucopyranoside.

The FABMS spectrum of **4** showed a peak at *m/z* 313 $[MH]^+$. As shown in Table 2, ^{13}C NMR spectrum of **4** exhibited six oxymethine signals showing the presence of inositol in addition to the 2-phenylpropionyl group. In ^{1}H NMR spectrum, the coupling constants of inositol moiety (J_{12} and J_{23} = 2.6 Hz, J_{34}, J_{45}, J_{56} and J_{16} = 9.5 Hz) indicated the inositol to be *myo*-inositol. A signal assigned to H-1 or H-5 shifted downfield (δ 4.60), indicating the 2-phenylpropionyl group to be attached to the C-1 or C-5 position of *myo*-inositol. The CD spectrum of **4** showed a negative maximum at 221 (Δε: − 1.83) and

*Part 59 in the series 'Studies on Plant Tissue Culture'. For Part 58, see Kawaguchi, K., Hirotani, M. and Furuya, T. (1989) *Phytochemistry* **28** (in press).

Table 1. ^{1}H NMR data for compounds **1**–**4** (300 MHz, CD_3OD)

H	1	2
2	3.82, 1H, *q* (7)*	‡
3	1.50, 1.5H, *d* (7), 1.47, 1.5H, *d* (7)	1.46, 1.14H, *d* (7), 1.47, 0.69H, *d* (7), 1.48, 0.69H, *d* (7), 1.49, 0.48H, *d* (7)
5, 6, 8, 9	7.28–7.34, 4H, *m*	§
7	7.20–7.26, 1H, *m*	§
1′	5.46, 0.5H, *d* (8), 5.47, 0.5H, *d* (8)	4.47, 0.38H, *d* (8), 4.64, 0.23H, *d*(8), 5.14, 0.23H, *d*, (3.8), 5.28, 0.16H, *d* (3.8)
2′	†	4.69, 0.61H, *dd* (9.5, 8), 4.58, 0.23H, *dd* (10, 3.8), 4.54, 0.16H, *dd* (10, 3.8)
3′	†	3.48, 0.61H, *t* (9.5), ‡
4′	†	3.35, 0.61H, *dd* (10, 9.5), 3.38, 0.39H, *dd* (10, 9)
5′	†	3.25, 0.61H, *ddd* (10, 6, 2.2), ‡
6′[a]	3.83, 0.5H, *dd* (11.5, 2), 3.77, 0.5H, *dd* (12, 1.8)	‡
6′[b]	3.68, 0.5 H, *dd* (11.5, 4.5), 3.62, 0.5H, *dd*(12, 4.5)	‡

H	3	4
2	3.93, 1H, *q* (7)	3.86, 1H, *q* (7)
3	1, 57, 3H, *d* (7)	1.50, 3H, *d* (7)
5, 6, 8, 9	7.38–7.45, 4H, *m*	‖
7	7.29–7.37, 1H, *m*	‖
1′	5.55, 1H, *d* (8)	4.60, 1H, *dd* (9.5, 2.6)[a]
2′	¶	4.06, 1H, *t* (2.6)
3′	¶	3.39, 1H, *dd* (9.5, 2.6)[a]
4′	¶	3.79, 1H, *t* (9.5)[b]
5′	3.84, 1H, *ddd* (8, 5, 2)	3.17, 1H, *t* (9.5)
6′[a]	4.11, 1H, *dd* (11.5, 2)	3.62, 1H, *t* (9.5)[b]
6′[b]	3.79, 1H, *dd*, (11.5, 5)	
1″	4.33, 1H, *d* (7.5)	
2″	3.25, 1H, *dd* (9, 7.5)	
3″	¶	
4″	3.56, 1H, *ddd* (10, 8.5, 5.2)	
5″[a]	3.89, 1H, *dd* (11.5, 5.2)	
5″[b]	3.20, 1H, *dd* (11.5, 10)	

*J in Hz shown in parentheses.
†3.20–3.45, 4H, *m*, ‡3.61–3.90, 3.78 H, *m*, §7.17–7.37, 5H, *m*, ‖7.20–7.37, 5H, *m*, ¶3.36–3.53, 4H, *m*.
[a,b]Assignments are interchangeable.

exhibited the reverse Cotton effect as **3** and (2*S*)-2-phenylpropionyl β-D-glucopyranoside indicating the configuration at C-2 of the 2-phenylpropionyl group of **4** to be *R*. Thus, **4** is a *myo*-inositol ester of (*R*)-2-phenylpropionic acid, but it has not been determined whether 2-phenylpropionic acid is attached to the C-1 or C-5 position.

Although **1** and **2** were mixtures of diastereoisomers with an (*RS*)-2-phenylpropionyl group at C-2, the configurations at C-2 of the 2-phenylpropionyl group of **3** and **4** were *S* and *R*, so that the primeverosyl ester of (*S*)-2-phenylpropionic acid and the *myo*-inositol ester of (*R*)-2-phenylpropionic acid, were formed stereoselectively.

To investigate the ability of root culture of *P. ginseng* to biotransform (*RS*)-2-phenylpropionic acid, a time course experiment was carried out. The root culture of *P. ginseng*, precultured for three weeks, was administered (*RS*)-2-phenylpropionic acid and harvested at days one to fourteen. The root culture converted (*RS*)-2-phenylpropionic acid into **1**–**4** with almost all of **2** and *ca* half of **1** being excreted into the medium. The conversion

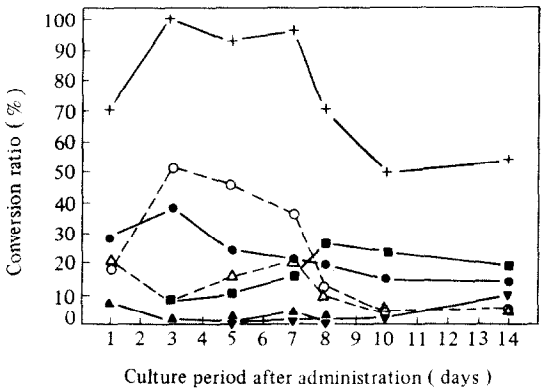

Fig. 1. Time course of the conversion products from 2-phenylpropionic acid: ●——●, **1** in the roots; ○---○, **1** in the medium; ▲——▲, **2** in the roots; △---△, **2** in the medium; ■——■, **3** in the roots ▼——▼, **4** in the roots; +——+, total convertion ratio.

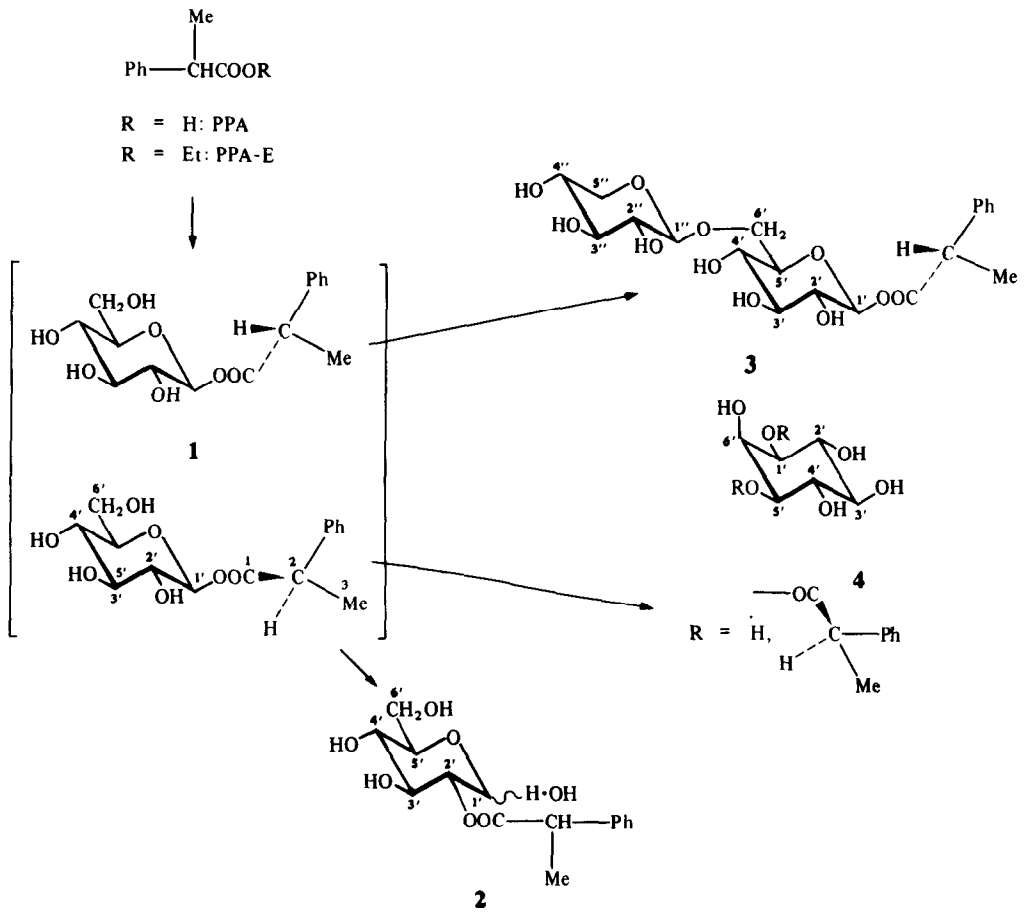

Fig. 2. Scheme for the biotransformation of 2-phenylpropionic acid by root cultures of *P. ginseng*.

into **1** and **2** were observed one day after administration, (Fig. 1) and the conversion into **2** was *ca* 20% and remained constant for seven days followed by a gradual decrease. The conversion into **1** was *ca* 44% at day one and the amount in the roots was greater than that excreted into the medium. However, at day three, the amount of **1** excreted into the medium was three-fold compared to 1.4-fold in the roots, and the amount in the medium increased more than that in the roots. After day three, the conversion into **1** in the roots decreased to *ca* 23% at day five, and then at day ten and thereafter the ratio remained constant as about 15%. The amount converted in the medium decreased gradually to about 35% at day seven, and thereafter to below 10%. The root culture may have absorbed **1** and converted it into other products. The conversion into **3** was observed at day three and increased with decreasing **1** to reach about 24% after day eight, thereafter decreasing gradually with time. The conversion into **4** was observed after day five but the ratio was low; thereafter the ratio increased gradually to about 8% at day fourteen. Compounds **3** and **4** accumulated in the roots and were not detected in the medium. The root culture of *P. ginseng* converts 2-phenylpropionic acid into **1** and excretes it into the medium. Then, **1** is absorbed in the cells and may be converted into **3** and **4**, which are more polar. Compound **3** may be formed selectively from the glucosyl ester of (*S*)-2-phenylpropionic acid and **4**, that of (*R*)-2-phenylpropionic acid, (Fig. 2). Compound **2** may be formed from **1** by non-enzymic acyl migration.

In order to compare the conversion abilities of the callus and root cultures of *P. ginseng*, the callus or root

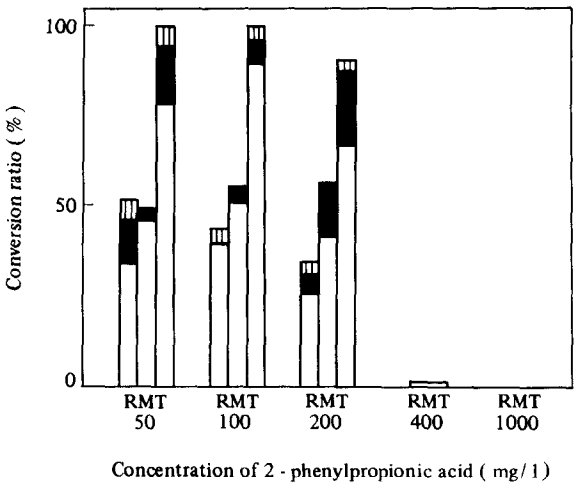

Fig. 3. Effect of substrate concentration administered to the medium on the conversion ratio: R, in the roots; M, in the medium; T, R + M; □, 1; ■, 2; ▥, 3.

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

C	1		2				3	4
1	175.3		175.8	176.4			175.2	176.4
2	46.9		46.8	47.0	47.1	47.2	46.8	47.0
3	19.5	19.7	19.6	19.7	19.9		19.6	19.6
4	141.8	141.8	142.2	142.3	142.5	142.6	141.9	142.4
5, 9	129.9[a]	130.0[a]	129.8[b]				130.0[c]	129.8[d]
6, 8	129.0[a]		128.9[b]	129.0[b]	129.1[b]		129.0[c]	129.0[d]
7	128.5		128.2	128.3			128.5	128.3
1′	96.3	96.4	91.3	91.4	96.6		96.3	76.4[e]
2′	74.2	74.3	76.4		77.0		74.1	71.9
3′	78.4	78.3	72.4		76.4		78.1	73.3[e]
4′	71.3	71.2	72.3		72.1		71.1	72.2[f]
5′	79.2		73.1	73.2	78.3	78.4	78.2	76.9
6′	62.6	62.5	62.9		63.0		69.5	74.3[f]
1″							105.4	
2″							75.1	
3″							77.8	
4″							71.4	
5″							67.1	

[a–f] Assignments are interchangeable.

For **1** and **2**, there are some columns for each carbon because **1** is a mixture of diastereoisomers of the 2-phenylpropionyl groups at C-2, and **2** is a mixture of α- and β-anomers of the glucose moiety and diastereoisomers of the 2-phenylpropionyl group at C-2.

culture of *P. ginseng*, precultured for three weeks, was administered 2-phenylpropionic acid or its ethyl ester and incubated for three days. Compared with the root culture showed the greatest glycosylation potential, the callus showed lower glycosylation ability (Table 3). The amount of **1** excreted into the medium was *ca* 25% and this showed low excretion from the callus culture. The low conversion ratio at 12.7% for ethyl 2-phenylpropionate in the root culture showed that this culture was not very successful at removing the ethyl grouping.

The effect of substrate concentration on the conversion ratio was examined. Concentrations of 2-phenylpropionic acid varying from 50 to 1000 mg/l were administered to the root culture, precultured for three weeks, and harvesting occurred three days later. At concentrations of 50 and 100 mg/l there was 100% conversion into products (Fig. 3). On administration of 200 mg/l, the conversion ratio was slight lower. By contrast, there was little or no observed conversion on administrations above 400 mg/l due to damage to the roots.

The root culture of *P. ginseng* has a high glycoslylation ability and *ca* a half the glucosyl ester is excreted into the medium, while the suspension culture of *Nicotiana tabacum* and *Dioscoreophyllum cumminsii* accumulated the same products in the cells [1].

EXPERIMENTAL

NMR spectra were determined in CD_3OD at 300 MHz. FABMS spectra was taken with a JEOL JMS D-300 instruments equipped with a direct inlet system.

Induction of root culture. A cell culture from petiole of two-year-old ginseng (*P. ginseng*) and subcultured on a Murashige–Skoog's medium containing 1 ppm 2,4-D, at 25° in the dark for 4 weeks [5, 8]. The callus was transferred onto Murashige–Skoog's medium containing 1 ppm kinetin under illumination (2500–4000 lux, 16 hr/day). This callus gradually generated rootlets and shoots. The rootlets were transferred and subcultured on a Murashige–Skoog's medium containing IBA 1 ppm in the dark, and they differentiated roots. The roots were transferred onto Murashige–Skoog's medium containing 5 ppm IBA and 0.1 ppm *N*-phenyl-*N*′-(4-pyridyl)urea, and subcultured for three weeks.

Induction of B2K callus. A cell culture was derived in 1978 from five-year-old ginseng root and subcultured on a Murashige-Skoog's medium containing 1 ppm 2,4-D and 0.1 ppm kinetin for 4 weeks. After a third subculture, the calli were transferred onto Murashige–skoog's medium containing 2 ppm IBA and 0.1 ppm kinetin (B2K) and subcultured for three weeks at 25° in the dark [9]. From the calli, white callus was selected and subcultured.

Method of administration. The callus and root cultures, which were cultured on agar media for 3 weeks, were transferred to the liquid media and cultured on a rotary shaker at 145 rpm and 25° in the dark. After three weeks, the test substrates, after being dissolved in 50% EtOH, were added to suspension cultures and cultured for one to fourteen days. The substrates used in this experiment were (*RS*)-2-phenylpropionic acid (colourless liquid, bp 264–265°) and (*RS*)-ethyl 2-phenylpropionate (colourless liquid, bp 230°), supplied by Nissan Chemical Industries Ltd.

Isolation of conversion products. The root culture previously administered 2-phenylpropionic acid was separated into medium and roots by filtration through nylon cloth. The roots were homogenized in MeOH, the homogenate was filtered. The filtrate was concd. dissolved in H_2O and combined with the medium. The solution applied to a column of Diaion HP-20 and washed with H_2O followed by elution with MeOH. The MeOH eluate was chromatographed on silica gel (WAKO gel C-200) using $CHCl_3$–MeOH (6:1) as the eluent. Three fractions were collected. From fraction 1, **1** was crystallized from *n*-hexane–EtOH. Compound **2** was separated from this mother liquor by HPLC. Compounds **3** and **4** were isolated from fraction 3 and 2, respectively, by HPLC. The HPLC column was Senshu-Pak ODS-4301-N(300 × 10 mm) and the eluents were MeOH–H_2O(3:7 for **2** and **3**, and 1:4 for **4**).

Table 3. Conversion ratio in callus and root culture for three day's incubation

	Substrate (100 mg/l)	Conversion ratio (%) Product 1			Product 2			Product 3		Total
		C	M	C+M	C	M	C+M	C	M	
Callus	PPA	27.9	9.8	37.7	—	—	—	—	—	37.7
Root	PPA	38.7	50.6	89.3	0.9	5.6	6.5	4.2	—	100.0
	PPA-E	3.9	3.7	7.6	2.7	1.8	4.5	0.6	—	12.7

PPA, 2-phenylpropionic acid; PPA-E, ethyl 2-phenylpropionate; C, in the callus or root; M, in the medium.
Product **4** was not produced during three day's incubation.

Quantitative analysis of conversion products. The media and MeOH extracts of the callus or 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 H_2O followed by elution with MeOH. The amount of each of the conversion products present in the MeOH eluate was determined by HPLC: Senshu-Pak ODS-4301-N column (300 × 10 mm), MeOH–H_2O(19:31), detection by differential refractometer and UV (254 nm) absorption. *Rt*(min) of **1–4** and 2-phenylpropionic acid were 16.9, 15.2, 14.5, 12.4 and 35.7, respectively. Ethyl 2-phenylpropionate was not detected in the eluent.

(2RS)-2-O-(2-*phenylpropionyl*)-D-*Glucose* (**2**). Amorphous solid: $[\alpha]_D^{24}$ + 20.8° (EtOH; *c* 1.18); IR ν_{max}^{KBr} cm^{-1}: 3410, 1715; ^1H NMR (CD_3OD): see Table 1; ^{13}C NMR (CD_3OD): see Table 2; FABMS *m/z*: 335 $[M + Na]^+$.

(2S)-2-*Phenylpropionyl* 6-O-β-D-*xylopyranosyl*-β-D-*glucopyranoside* (**3**). Amorphous solid: $[\alpha]_D^{24}$ − 5.5° (EtOH; *c* 1.09); IR ν_{max}^{KBr} cm^{-1}: 3405, 1740; ^1H NMR (CD_3OD): see Table 1; ^{13}C NMR (CD_3OD): see Table 2; FABMS *m/z*: 467 $[M + Na]^+$; **4**. CD(EtOH; *c* 3.69×10^{-4}) $\Delta\varepsilon^{19}$: +4.43 (221) (pos. max). Amorphous solid: $[\alpha]_D^{24}$ − 40.0° (EtOH; *c* 0.64); IR ν_{max}^{KBr} cm^{-1}: 3350, 1710; ^1H NMR (CD_3OD): see Table 1; ^{13}C NMR: see Table 2; FABMS *m/z*: 313 $[MH]^+$; CD(EtOH; *c* 2.82×10^{-4}) $\Delta\varepsilon^{19}$: −1.81 (221) (neg. max).

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