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BIOTRANSFORMATION OF (*RS*)-TROPIC ACID IN SUSPENSION CULTURES OF *COFFEA ARABICA, DATURA INNOXIA, EUCALYPTUS PERRINIANA* AND *NICOTIANA TABACUM**

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Key Word Index—*Coffea arabica, Datura innoxia, Eucalyptus perriniana, Nicotiana tabacum*; cell suspension culture, biotransformation; glycosylation; (*RS*)-tropic acid; (*RS*)-2-(4-hydroxyphenyl)propionic acid; (*R*)-tropic acid isotrehalose ester.

Abstract—Suspension cultures of *Datura innoxia* and *Nicotiana tabacum* are able to convert (*RS*)-tropic acid into its glucose esters (2*RS*)-3-hydroxy-2-phenylpropionyl β-D-glucopyranoside and (2*RS*)-2-*O*-(3-hydroxy-2-phenylpropionyl)-D-glucose whereas a cultures of *Eucalyptus perriniana* converts it into its glucoside (2*RS*)-3-*O*-β-D-glucopyranosyl-2-phenylpropionic acid in addition to glucose esters. Suspension cultures of *Coffea arabica* converts: (*RS*)-tropic acid into its glucose, sucrose and isotrehalose esters and a small amount of its glucoside; (*RS*)-2-(4-hydroxyphenyl)propionic acid into its glucose and sucrose esters and a small amount of its glucoside; and (*RS*)-ethyl 2-(4-hydroxyphenyl)propionate into its gentiobioside. The formation of sucrose esters and linkage of the aglycone to the C-6 position of glucose are characteristic of the biotransformation of carboxylic acids by suspension cultures of *C. arabica*. The suspension culture of *C. arabica* selectively converted (*R*)-tropic acid into its isotrehalose ester on administration of (*RS*)-tropic acid.

INTRODUCTION

We have investigated the biotransformation of phenylpropanoids and their analogues by plant cell cultures. In our previous papers, we reported on the biotransformation of 2-phenylpropionic acid in suspension cultures of *Coffea arabica* [1] and *Nicotiana tabacum* [2]. The culture of *C. arabica* mainly converted 2-phenylpropionic acid into its sucrose ester, and that of *N. tabacum* into its glucose and gentiobiose esters. The plant of *Datura innoxia* produces tropane alkaloids such as hyoscyamine and scopolamine. Suspension cultures of *D. innoxia* have been reported to glucosylate phenolic compounds such as hydroquinone [3] and salicyl alcohol [4]. A suspension culture of *Eucalyptus perriniana* was observed to hydroxylate and glycosylate *l*-menthol [5]. The glycosylation step gave rise to gentiobiosides binding to the alcoholic hydroxy group of *l*-menthol.

Tropic acid is a component of tropane alkaloids and a hydroxylated derivative of 2-phenylpropionic acid. The present paper reports on the biotransformation of tropic acid having a alcoholic hydroxy group in suspension cultures of *C. arabica, D. innoxia, E. perriniana* and *N. tabacum*. A suspension culture of *C. arabica* was used to investigate the biotransformation of 2-(4-hydroxyphenyl)propionic acid having a phenolic hydroxy group and its ethyl ester.

*Part 62 in the series 'Studies on Plant Tissue Culture'. For part 61, see Furuya, T., Orihara, Y. and Miyatake, H. (1989) *J. Chem. Soc., Perkin I* (in press).

RESULTS AND DISCUSSION

Compounds **1**–**3** were isolated from cultured cells of *D. innoxia* and *N. tabacum* previously administered (*RS*)-tropic acid (Fig. 1). The ^{1}H and ^{13}C NMR spectra of **1**–**3** were in agreement with those of (2*S*)-3-hydroxy-2-phenylpropionyl β-D-glucopyranoside, (2*R*)-3-hydroxy-2-phenylpropionyl β-D-glucopyranoside and (2*RS*)-2-*O*-(3-hydroxy-2-phenylpropionyl)-D-glucose, respectively. All of these compounds have been isolated from a root culture of *Panax ginseng* previously administered (*RS*)-tropic acid [6]. From the suspension culture of *E. perriniana*, **4** and **5** in addition to **1** and **3** were isolated (Fig. 1). The ^{1}H and ^{13}C NMR spectra of **4** and **5** were in agreement with those of (2*S*)-3-*O*-β-D-glucopyranosyl-2-phenylpropionic acid and (2*R*)-3-*O*-β-D-glucopyranosyl-2-phenylpropionic acid, respectively. These compounds also have been isolated from a root culture of *P. ginseng* previously administered (*RS*)-tropic acid [6].

Suspension cultures of *D. innoxia* and *N. tabacum* converted salicyl alcohol into isosalicin in which salicyl alcohol is linked to glucose at the alcoholic hydroxy group [4]. However, the same cultures converted tropic acid, which also contains an alcoholic hydroxy group, mainly to its glucose esters. This result indicated that group selectivity on glycosylation changes in response to the combination of functional groups. The formation of glucosides **4** and **5** in suspension cultures of *E. perriniana* showed that the alcoholic hydroxy group of tropic acid can be glycosylated though the selectivity between alcoholic hydroxy and carboxylic groups was low.

Compounds **6**–**10** were isolated from cultured cells of *C. arabica* previously administered (*RS*)-tropic acid.

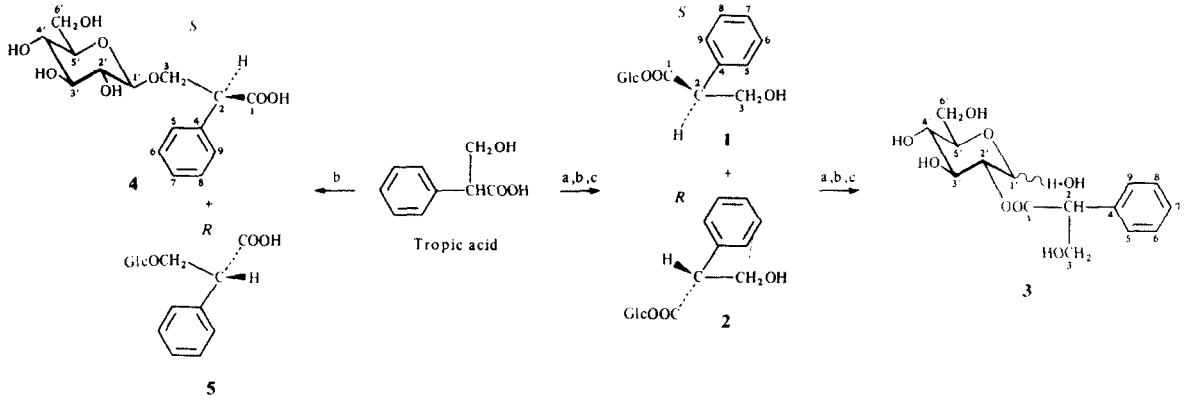

Fig. 1. Possible scheme for the biotransformation of (*RS*)-tropic acid by suspension cultures of *D. innoxia* (a), *E. perriniana* (b) and *N. tabacum* (c).

Compounds **4** and **5** were isolated as minor products (Fig. 2). The FABMS spectra of **6** and **7** showed peaks at *m/z* 351 $[M+Na]^+$. The ^1H and ^{13}C NMR spectra of **6** and **7** showed the presence of the tropoyl group and α- and β-anomers of glucose units respectively (Experimental and Table 1). In the ^1H NMR, acylation shifts (about $\delta+0.7$) at the H-6 of the glucose units indicated that the tropoyl groups are attached to the glucose units at C-6. The chemical shifts between **6** and **7** differed slightly. The difference possibly arises from the differences in the configurations at C-2 of the tropoyl groups. The CD spectrum of **6** showed a negative maximum at 218 ($\Delta\varepsilon: -2.60$) and that of **7**, a positive maximum at 218 ($\Delta\varepsilon: +1.06$), indicating the configuration at C-2 of the tropoyl group of **6** may to be *S* and that of **7**, *R*.

The FAB MS spectra of **8**–**10** each showed a peak at *m/z* 513 $[M+Na]^+$, which was larger by 162 mass units than that of **6** and **7**, indicating the presence of an additional hexose group. The ^1H and ^{13}C NMR spectra of the sugar moieties of **8** and **9** were in good agreement with those of sucrose [1, 7–9] after allowance for acylation at the C-6 position of the glucose residue of sucrose. The CD spectrum of **8** showed a negative maximum at 219 ($\Delta\varepsilon: -6.24$) and **9**, a positive maximum at 218 ($\Delta\varepsilon: +3.00$) indicating the configuration at C-2 of the tropoyl group of **8** may to be *S* and **9**, *R*. A comparison of the ^{13}C NMR spectrum of **10** with that of the β-anomer of **7** revealed an additional set of signals due to a terminal β-glucosyl unit in **10** (Table 1). The chemical shifts of the anomeric carbons of the glucose moieties were δ 100.5 and 100.8 and the signals assignable to the C-2 positions of these moieties (δ 75.0 and 74.9) were shifted by $\delta-1.5$ and -1.6 compared with the β-anomer of **7** indicating the terminal glucose to be linked to the anomeric position of the inner glucose. The ^{13}C NMR spectrum of the sugar moiety of **10** was in good agreement with that of isotrehalose (β,β-trehalose) [7, 8] after allowance for the acylation at the C-6 position of inner glucose moiety. In the ^1H NMR spectrum of **10**, the large coupling constants ($J=7.9$ Hz) of the anomeric protons suggest the configurations of the anomeric centres to be β. The ^1H NMR spectrum also showed the structure of **10** to be that of the isotrehalose ester of tropic acid (Experimental). The CD spectrum of **10** showed a positive maximum at 218 ($\Delta\varepsilon$: +2.08) indicating the configuration at C-2 of the tropoyl group in **10** may to be *R*.

From these results **6** was assigned the structure of (2*S*)-6-*O*-(3-hydroxy-2-phenylpropionyl)-D-glucose, **7** of (2*R*)-6-*O*-(3-hydroxy-2-phenylpropionyl)-D-glucose, **8** of (2*S*)-β-D-fructofranosyl 6-*O*-(3-hydroxy-2-phenylpropionyl)-α-D-glucopyranoside, **9** of (2*R*)-β-D-fructofranosyl 6-*O*-(3-hydroxy-2-phenylpropionyl)-α-D-glucopyranoside and **10** of (2*R*)-β-D-glucopyranosyl 6-*O*-(3-hydroxy-2-phenylpropionyl)-β-D-glucopyranoside. The configurations at C-2 of the tropoyl groups of **6**–**10** were estimated by comparison of their CD spectra with that of (2*S*)-2-phenylpropionyl β-D-glucopyranoside prepared from (*S*)-2-phenylpropionic acid by biotransformation using a root culture of *Panax ginseng*. Compared with 2-phenylpropionic acid, tropic acid has a hydroxy group at C-3 and as a result the Cotton effects may be reversed. Thus the configurations at C-2 of the tropoyl groups of **6**–**10** may be reversed.

Compounds **11**–**13** were isolated from the suspension culture of *C. arabica* previously administered 2-(4-hydroxyphenyl)propionic acid, and **14** from that previously administered the ethyl ester of 2-(4-hydroxyphenyl)-propionic acid [ethyl 2-(4-hydroxyphenyl)propionate] (Fig. 3). Compound **11** was a minor conversion product and its ^1H and ^{13}C NMR spectra were in agreement with those of (2*RS*)-2-(4-*O*-β-D-glucopyranosylphenyl)propionic acid previously isolated from root culture of *P. ginseng* administered 2-(4-hydroxyphenyl)propionic acid [6]. The FAB MS spectrumof **12** showed a peak at *m/z* 351 $[M+Na]^+$. As shown in Table 1 and the Experimental, the ^{13}C and ^1H NMR spectra showed **12** to be a mixture of diastereoisomers in which the signals of the sugar moiety were in good agreement with those of **6** and **7** indicating that the 2-(4-hydroxyphenyl)-propionyl group was attached to C-6 of the glucose moiety. The FABMS spectrum of **13** showed a peak at *m/z* 513 which was larger by 162 mass units than that of **12**. The ^1H and ^{13}C NMR spectra showed **13** to be a mixture of diastereoisomers with the same sugar moiety as **8** and **9**. This indicated that the 2-(4-hydroxyphenyl)propionyl group was attached to C-6 of the glucose residue of a sucrose moiety. The FABMS spectrum of **14** showed a peak at *m/z* 541 $[M+Na]^+$. The

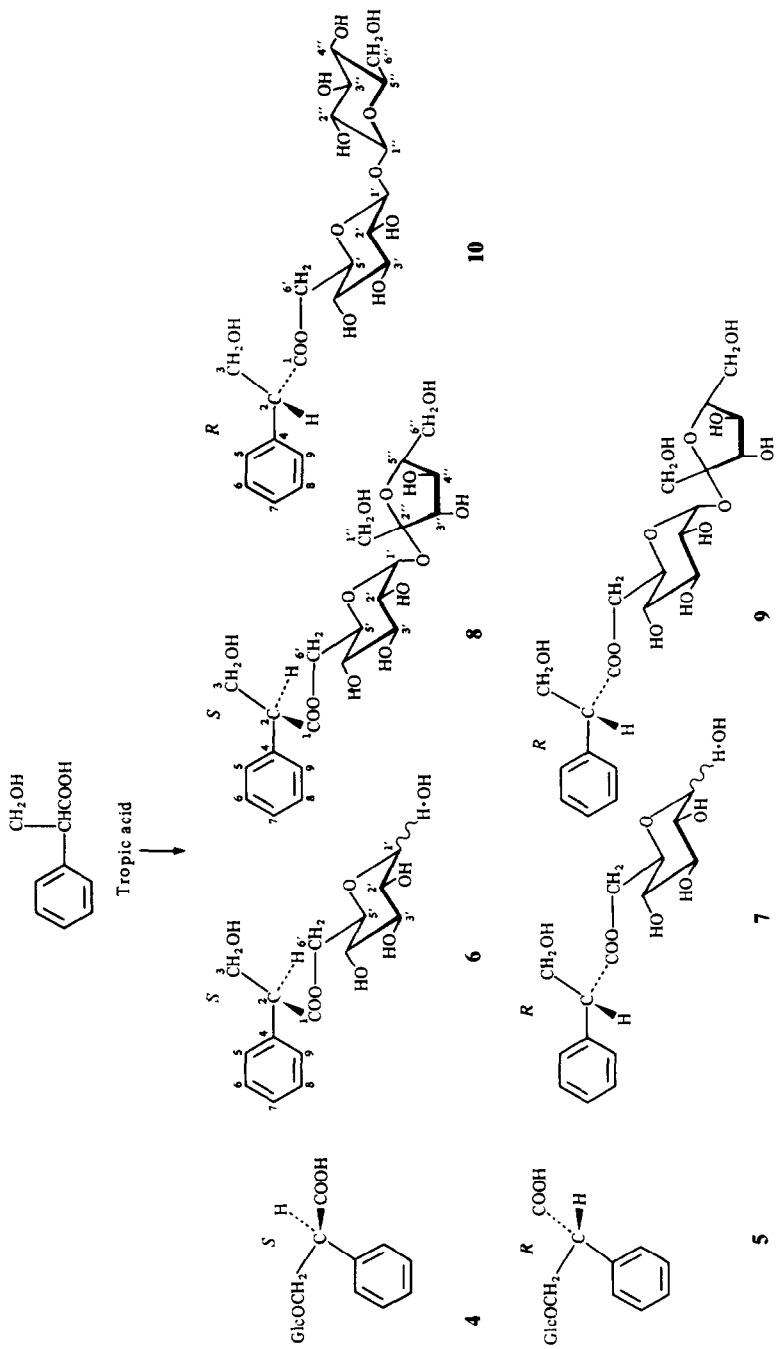

Fig. 2. Biotransformation of (*RS*)-tropic acid by a suspension culture of *C. arabica*.

Table 1. ^{13}C NMR data for compounds **6**–**10**, **12**–**14** (75 MHz, CD_3OD)

C	6	7	8	9	10	12	13	14
1	174.5 174.4	174.4	174.5	174.5	174.4	177.0	177.0	176.6
2	56.2	56.4	55.9	56.2	56.3	46.3 46.2 46.1	46.1 46.0	46.2
3	65.6[a] 65.4[a]	65.6[c] 65.5[c]	65.4	65.6	65.5[g]	19.5 19.4	19.6 19.4	19.4
4	137.7 137.6	137.7 137.6	137.6	137.6	137.6	133.1	133.2 133.1	136.1
5,9	130.0[b]	130.2[d]	130.0[e]	130.0[f]	130.2[h]	129.8	129.9	129.7
6,8	129.6[b]	129.7[d] 129.6[d]	129.6[e]	129.6[f]	129.6[h]	116.6	116.6	118.0
7	128.9	128.9	129.0	129.0	129.0	157.9	157.9	158.1
1′	94.2 98.5	94.3 98.5	93.7	93.7	100.5[i]	94.2 98.5	93.8	102.4 102.3
2′	73.9 76.4	74.0 76.5	73.2	73.4	75.0[j]	74.0 76.5	73.4	75.1[l]
3′	74.9 78.2	75.0 78.2	74.6	74.8	78.1	75.0 78.2	74.8 74.7	77.1[m]
4′	71.9 71.1	72.0 71.0	71.5	71.9	71.8[k]	72.0 71.9 71.2 71.1	71.7	71.8[n]
5′	72.0 75.7	72.2 75.7	72.4	72.4	75.8	72.1 75.8	72.5	78.0[m]
6′	65.2[a] 65.6[a]	65.6[c]	64.9	65.3	65.4[g]	65.5 65.4 65.3 65.1	64.9 64.8	70.0
1″			64.3	64.4	100.8[i]		64.4	104.9
2″			105.5	105.5	74.9[j]		105.6	75.4[l]
3″			79.5	79.5	78.1		79.6	77.6[m]
4″			76.2	76.2	71.7[k]		76.3 76.2	71.5[n]
5″			84.1	84.1	78.4		84.2	78.2
6″			64.1	64.2	62.9		64.2 64.1	63.0[p]
OCH_2								62.2[p]
Me								14.8

[a–p] Assignments are interchangeable.

For **6**, **7**, **12**, **13** and **14** there are several columns for each carbon because **6** and **7** are mixtures of α- and β-anomers of the glucose moiety, **12** is a mixture of diastereoisomers on C-2 of the 2-(4-hydroxyphenyl)propionyl group and α- and β-anomers of the glucose moiety and **13** and **14** are mixtures of diastereoisomers on C-2 of the 2-(4-hydroxyphenyl)propionyl group.

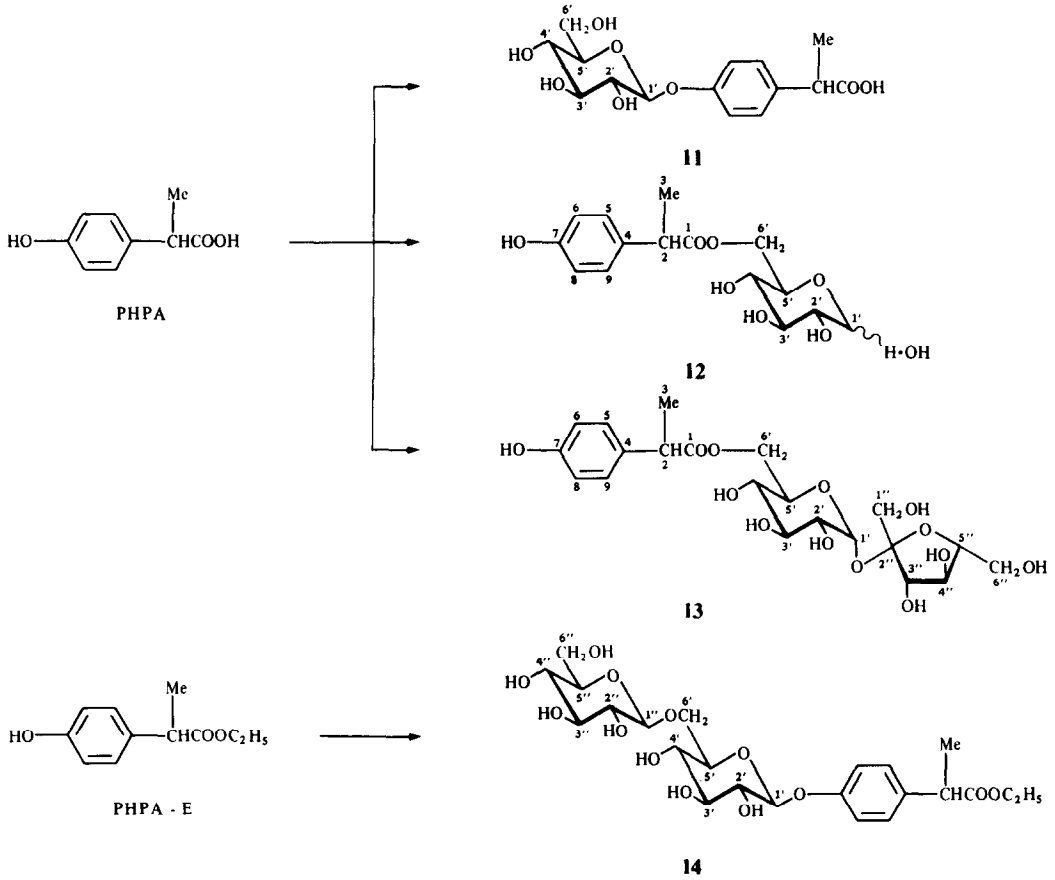

Fig. 3. Biotransformation of (*RS*)-2-(4-hydroxyphenyl) propionic acid (PHPA) and its ethyl ester (PHPA-E) by a suspension culture of *C. arabica*.

^{13}C NMR spectrum of **14** showed the presence of an ethyl group and an additional β-glucosyl unit compared with that of **11**. The glycosylation shifts at C-6 ($\delta + 7.2$) of the inner glucose moiety of **14** indicated that the terminal glucose in **14** is linked to C-6 of the inner glucose. In the ^{1}H NMR spectrum of **14**, the presence of two sets of peaks for the anomeric proton of the glucose moiety showed **14** to be a mixture of diastereoisomers. An acylation shift of a methylene proton carrying an oxygen function indicated the ethyl group to be acylated by 2-(4-hydroxyphenyl)propionyl group. From these results **12** was assigned the structure of (2*RS*)-6-*O*-[2-(4-hydroxyphenyl)propionyl]-D-glucose, **13** of (2*RS*)-β-D-fructofuranosyl 6-*O*-[2-(4-hydroxyphenyl)propionyl]-α-D-glucopyranoside, and **14** of (2*RS*)-ethyl 2-[4-*O*-(6-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)phenyl]propionate.

In the biotransformation of 2-(4-hydroxyphenyl)propionic acid and tropic acid by the suspension culture of *C. arabica*, although small amounts of their glucosides (**4**, **5** and **11**) were produced, almost all the products were sugar esters (**6**–**10**, **12** and **13**) (Figs 2 and 3). Therefore suspension culture of *C. arabica* selectively glycosylate the carboxylic group for the substrates having both carboxylic and hydroxy groups. However, on administration of ethyl 2-(4-hydroxyphenyl)propionate, deethylation was not observed and a gentiobioside was produced.

The suspension culture of *N. tabacum* converted 2-phenylpropionic acid into its glucose and gentiobiose esters [2], and tropic acid, into its mono glucose esters. The suspension cultures of *D. innoxia* and *E. perriniana* and the root culture of *Panax ginseng* [6] also converted tropic acid into products bound to only one glucose. On the other hand, the suspension culture of *C. arabica* converted tropic acid into sucrose and isotrehalose esters (**8**–**10**) in addition to glucose esters (**6** and **7**), and on administration of 2-(4-hydroxyphenyl)propionic acid also, the sucrose ester (**13**) was mainly produced in addition to the glucose ester (**12**). However, the production of isotrehalose ester was not observed. On administration of tropic acid and 2-(4-hydroxyphenyl)propionic acid to the suspension culture of *C. arabica*, the carboxylic acids were linked to C-6 of glucose. Conversion into sucrose ester was also observed on administration of phenylacetic acid and 2-phenylpropionic acid, in a previous paper [1] as well as the carboxylic acid linked to C-6 of glucose. The suspension culture of *C. arabica* has the ability to convert phenylcarboxylic acids into sucrose esters in which C-6 of the glucose residue of sucrose is involved in the ester bond. The ester linkage to C-6 of a glucose moiety are characteristic of the biotransformation of carboxylic acid by suspension cultures of *C. arabica*. The configuration at C-2 of the isotrehalose ester (**10**) of tropic acid was *R* and the isotrehalose ester of (*S*)-tropic acid has not been observed.

Although the conversion into glucose and sucrose esters (**6**–**9**) was not selective with regard to the configuration at C-2 of (*RS*)-tropic acid, the conversion into isotrehalose esters may be selective. Isotrehalose was observed in starch hydrolyses, but isotrehalose and its derivative have not been observed in plants and plant cell and tissue cultures. Sugars binding administered substrates and their binding points seems to relate to the components of plants and their cell and tissue cultures. Although sucrose and isotrehalose esters have not been identified from the plant and the cell culture of *C. arabica*, they may contain sucrose and isotrehalose and/or their esters and/or have ability to produce sucrose and isotrehalose esters in response to stimulation such as substrate administration.

EXPERIMENTAL

NMR: CD_3OD. FABMS was taken with a JEOL JMS DX-300 instruments equipped with a direct inlet system.

Culture and administration method. The cell cultures of *Coffea arabica* [1], *Datura innoxia* and *Nicotiana tabacum* [2, 10] were initiated in 1982, 1969 and 1966, respectively, from seed, seedling and stem, respectively, and subcultured on a 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. The cell culture of *Eucalyptus perriniana* initiated in 1980 from stem, and subcultured on a Murashige and Skoog's agar medium containing 6-benzylaminopurine (1 ppm) at 25° in the dark for 3 weeks. These calli were transferred to a liquid medium of the same composition as the subculture medium, and cultured on a rotary shaker at 145 rpm and 25° in the dark. After 2–3 weeks, 50 mg of (*RS*)-tropic acid(purchased from Wako Pure Chemical) dissolved in 2 ml H_2O or 25 mg of (*RS*)-2-(4-hydroxyphenyl)propionic acid or its ethyl ester (colourless liquid, bp 110–117°/5 mmHg; supplied by Nissan Chemical Industries Ltd) dissolved in 2 ml 50% EtOH was added to 250 ml of each suspension culture and cultured for 3 days. (*RS*)-2-(4-hydroxyphenyl)propionic acid was prepared from its ethyl ester by KOH hydrolysis.

Isolation of conversion products from the suspension cultures of D. innoxia *and* N. tabacum. The suspension culture of *D. innoxia* (1250 ml) previously administered tropic acid was separated into medium and cells by filtration through Nylon cloth, and the latter homogenized with MeOH. The homogenate was filtered, concd and suspended in H_2O. The suspension was applied to a column of Diaion HP-20 and washed with H_2O followed by elution with MeOH. The MeOH eluate was chromatographed on a column of silica gel (Wako gel C-200) using $CHCl_3$–MeOH–H_2O (6:4:1) as the eluent. From the eluent, compounds **1** (3.2 mg), **2** (3.6 mg) and **3** (1.4 mg) were isolated by HPLC using a Senshu Pak ODS-4301-N column and MeOH–H_2O (1:4) as the eluent. From the suspension culture of *N. tabacum* (3 1), compounds **1** (71.5 mg), **2** (31.3 mg) and **3** (32.3 mg) were isolated by a similar method to that just described for *D. innoxia*.

Isolation of conversion products from the suspension culture of E. perriniana. The suspension culture of *E. perriniana* previously administered tropic acid (total 400 mg) was sepd into medium and the cells, and the latter homogenized with MeOH. The homogenate was filtered, concd and combined with the medium, and applied to a column of Diaion HP-20. The column was washed with H_2O and eluted with MeOH. The eluate was chromatographed on a column of silica gel using $CHCl_3$–MeOH (4:1) as the eluent. From the eluate, compounds **1** (10.4 mg), **3** (12.0 mg), **4** (7.1 mg) and **5** (8.8 mg) were isolated by HPLC using a Senshu Pak ODS-4301-N column and MeOH–H_2O (1:4) as the eluent.

Isolation of compounds **6**–**10**. The MeOH eluate of a HP-20 column prepared from the cultured cells of *C. arabica* previously administered tropic acid (total 700 mg) by the same method as the isolation of products from *D. innoxia*, was chromatographed on a column of silica gel using $CHCl_3$–MeOH–H_2O (25:5:2, lower layer) as the eluent. Fraction 1 and 2 were collected. From fraction 1, compounds **6** (11.2 mg) and **7** (26.1 mg) were isolated by HPLC using a Senshu Pak ODS-4301-N column and MeOH–H_2O (1:4) as the eluent and compounds **4**, **5** (**4**+**5**, 5.2 mg), **8** (110.1 mg), **9** (134.0 mg) and **10** (45.8 mg), from fraction 2.

Isolation of compounds **11**–**13**. Compounds **11** (2.1 mg), **12** (7.6 mg) and **13** (17.0 mg) were isolated from the medium and the cells of *C. arabica* previously administered 2-(4-hydroxyphenyl)propionic acid (total 250 mg) by a similar method to that used to isolate a **4**–**10**.

Isolation of compound **14**. The suspension culture of *C. arabica* previously administered ethyl 2-(4-hydroxyphenyl)propionate (total 100 mg) was separated into medium and cells, and the latter was homogenized with MeOH. The homogenate was filtered, concentrated and combined with the medium, and extracted with EtOAc. The aq. layer was extracted with *n*-BuOH saturated with H_2O. The *n*-BuOH extract was chromatographed on a column of silica gel using $CHCl_3$–MeOH (7:3) as the eluent. From the eluate, compound **14** (45 mg) was isolated by HPLC using Senshu Pak AQUASIL SS826Y and ODS-4301-N columns and $CHCl_3$–MeOH–H_2O (7:3:1, lower layer) and MeOH–H_2O (2:3), respectively, as the eluents.

(2S)-6-O-(3-*hydroxy*-2-*phenylpropionyl*)-D-*Glucose* (**6**). Amorphous solid: $[\alpha]_D^{22}$ 32.7° (MeOH; *c* 0.99); IR ν_{max}^{KBr} cm^{-1}: 3420, 1725; ^1H NMR (CD_3OD): δ3.82 (0.6 H, *dd*, *J* = 9, 5.5 Hz) and 3.81 (0.4H, *dd*, *J* = 9, 5.5 Hz, H-2), 3.995 (0.6 H, *dd*, *J* = 10.5, 9 Hz) and 3.990 (0.4 H, *dd*, *J* = 10.5, 9 Hz, H-3), 3.72 (1H, *dd*, *J* = 10.5, 5.5 Hz, H-3), 4.99 (0.6H, *d*, *J* = 3.8 Hz) and 4.44 (0.4H, *d*, *J* = 7.8 Hz, H-1'), 3.21 (0.6H, *dd*, *J* = 9.5, 3.8 Hz) and 3.10 (0.4H, *dd*, *J* = 8.5, 7.8 Hz, H-2'), 3.62 (0.6H, *dd*, *J* = 9.5, 9.5 Hz, H-3'), 3.89 (0.6H, *ddd*, *J* = 10, 5, 2 Hz) and 3.43 (0.4H, *ddd*, *J* = 9.5, 6, 2 Hz, H-5'), 4.41 (0.6H, *dd*, *J* = 12, 2 Hz), 4.405 (0.4H, *dd*, *J* = 12, 2 Hz), 4.263 (0.6H, *dd*, *J* = 12, 5 Hz) and 4.262 (0.4H, *dd*, 12, 6 Hz, H-6'), 7.29–7.37 (4H, *m* H-5, 6, 8 and 9), 7.24–7.29 (1H, *m*, H-7), 3.23–3.32 (1.4H, *m*, H-3' and 4'); ^{13}C NMR (CD_3OD): see Table 1; FABMS *m/z* 351 $[M+Na]^+$; CD (EtOH; *c* 5.12×10^{-4}) $\Delta\varepsilon^{19}$: −2.60 (218) (neg. max).

(2R)-6-O-(3-*hydroxy*-2-*phenylpropionyl*)-D-*Glucose* (**7**). Amorphous solid: $[\alpha]_D^{22}$ 45.0° (MeOH; *c* 1.63); IR ν_{max}^{KBr} cm^{-1}: 3405, 1730; ^1H NMR (CD_3OD): δ3.82 (1H; *dd*, *J* = 9, 5.5 Hz, H-2), 4.090 (0.4H, *dd*, *J* = 10.5, 9 Hz) and 4.085 (0.6H, *dd*, *J* = 10.5, 9 Hz, H-3), 3.730 (0.6H, *dd*, *J* = 10.5, 5.5 Hz) and 3.725 (0.4H, *dd*, *J* = 10.5, 5.5 Hz, H-3), 5.05 (0.6H, *d*, *J* = 3.8 Hz) and 4.45 (0.4H, *d*, *J* = 7.6 Hz, H-1'), 3.30 (0.6H, *dd*, *J* = 9.5, 3.8 Hz) and 3.10 (0.4H, *dd*, *J* = 9.5, 7.6 Hz, H-2'), 3.65 (0.6H, *dd*, *J* = 9.5, 9 Hz, H-3'), 3.24 (0.6H, *dd*, *J* = 10, 9 Hz, H-4'), 3.32 (0.4H, *dd*, *J* = 9.5, 9 Hz, H-3' or 4'), 3.27 (0.4H, 5, 9 Hz, H-3' or 4'), 3.95 (0.6H, *ddd*, *J* = 10, 5, 2 Hz) and 3.47 (0.4H, *ddd*, *J* = 9.5, 6, 2 Hz, H-5'), 4.46 (0.6H, *dd*, *J* = 11.8, 2 Hz), 4.52 (0.4H, *dd*, *J* = 11.8, 2 Hz), 4.18 (0.6H, *dd*, *J* = 11.8, 5 Hz) and 4.15 (0.4H, *dd*, *J* = 11.8, 6 Hz, H-6'), 7.28–7.39 (4H, *m*, H-5, 6, 8 and 9), 7.23–7.28 (1H, *m*, H-7); ^{13}C NMR (CD_3OD): see Table 1; FABMS *m/z*: 351 $[M+Na]^+$; CD (EtOH; *c* 4.57×10^{-4}) $\Delta\varepsilon^{19}$: 1.06 (218) (pos. max).

(2S)-β-D-*Fructofuranosyl* 6-O-(3-*hydroxy*-2-*phenylpropionyl*)-α-D-*glucopyranoside* (**8**). Amorphous solid: $[\alpha]_D^{21}$ 28.4° (MeOH; *c* 1.09); IR ν_{max}^{KBr} cm^{-1}: 3420, 1735; ^1H NMR (CD_3OD): δ3.87 (1H, *dd*, *J* = 9, 5.5 Hz, H-2), 4.11 (1H, *dd*, *J* = 10.5, 9 Hz, H-3), 3.72 (1H, *dd*, *J* = 10.5, 5.5 Hz, H-3), 5.27 (1H, *d*, *J* = 3.8 Hz, H-1'), 3.25 (1H, *dd*, *J* = 9.5, 3.8 Hz, H-2'), 3.66 (1H, *dd*, *J* = 9.5, 9 Hz, H-3'), 3.24 (1H, *dd*, *J* = 10, 9 Hz, H-4'), 3.96 (1H, *ddd*, *J* = 10, 4.5, 2 Hz,

H-5'), 4.41 (1H, *dd*, $J = 12$, 2 Hz) and 4.29 (1H, *dd*, $J = 12$, 4.5 Hz, H-6'), 3.61 (1H, *d*, $J = 12$ Hz) and 3.56 (1H, *d*, $J = 12$ Hz, H-1''), 4.09 (1H, *d*, $J = 8$ Hz, H-3''), 3.98 (1H, *t*, $J = 8$ Hz, H-4''), 7.30–7.38 (4H, *m*, H-5, 6, 8 and 9), 7.24–7.29 (1H, *m*, H-7), 3.76–3.80 (3H, *m*, H-5'' and 6''); ^{13}C NMR (CD_3OD): see Table 1; FABMS *m/z*: 513 $[M+Na]^+$; CD (EtOH; $c\,4.37 \times 10^{-4}$) $\Delta\varepsilon^{19}$: −6.24 (219) (neg. max).

(2R)-*β*-D-*Fructofuranosyl* 6-O-(3-*hydroxy*-2-*phenylpropionyl*)-α-D-*glucopyranoside* (**9**). Amorphous solid: $[\alpha]_D^{22}$ 53.8° (MeOH; *c* 1.28); IR ν_{max}^{KBr} cm^{-1}: 3420, 1730; ^{1}H NMR (CD_3OD): *δ* 3.87 (1H, *dd*, $J = 9$, 5.5 Hz, H-2), 4.11 (1H, *dd*, $J = 10.5$, 9 Hz, H-3), 3.73 (1H, *dd*, $J = 10.5$, 5.5 Hz, H-3), 5.35 (1H, *d*, $J = 3.8$ Hz, H-1'), 3.36 (1H, *dd*, $J = 9.5$, 3.8 Hz, H-2'), 3.69 (1H, *dd*, $J = 9.5$, 9 Hz, H-3'), 3.25 (1H, *dd*, $J = 9.5$, 9 Hz, H-4'), 4.03 (1H, *ddd*, $J = 9.5$, 5, 2 Hz, H-5'), 4.58 (1H, *dd*, $J = 12$, 2 Hz) and 4.10 (1H, *dd*, $J = 12$, 5 Hz, H-6'), 3.61 (1H, *d*, $J = 12$ Hz) and 3.57 (1H, *d*, $J = 12$ Hz, H-1''), 4.09 (1H, *d*, $J = 8$ Hz, H-3''), 4.00 (1H, *t*, $J = 8$ Hz, H-4''), 7.30–7.38 (4H, *m*, H-5, 6, 8 and 9), 7.23–7.30 (1H, *m*, H-7), 3.74–3.80 (3H, *m*, H-5'' and 6''); ^{13}C NMR (CD_3OD): see Table 1; FABMS *m/z*: 513 $[M+Na]^+$; CD (EtOH; $c\,3.43 \times 10^{-4}$) $\Delta\varepsilon^{19}$: 3.00 (218) (pos. max).

(2R)-*β*-D-*Glucopyranosyl* 6-O-(3-*hydroxy*-2-*phenylpropionyl*)-*β*-D-*glucopyranoside* (**10**). Amorphous solid: $[\alpha]_D^{24}$ −15.4° (MeOH; *c* 1.14); IR ν_{max}^{KBr} cm^{-1}: 3420, 1735; ^{1}H NMR (CD_3OD): *δ* 3.93 (1H, *dd*, $J = 9$, 5.5 Hz, H-2), 4.20 (1H, *dd*, $J = 10.5$, 9 Hz, H-3), 3.84 (1H, *dd*, $J = 10.5$, 5.5 Hz, H-3), 4.80 (1H, *dd*, $J = 7.9$ Hz, H-1' or 1''), 3.48 (1H, *dd*, $J = 9$, 7.9 Hz, H-2' or 2''), 3.47 (1H, *t*, $J = 9$ Hz, H-3' or 3''), 3.61 (1H, *ddd*, $J = 9.5$, 5.8, 2 Hz, H-5'), 4.60 (1H, *dd*, $J = 11.8$, 2 Hz) and 4.31 (1H, *dd*, $J = 11.8$, 5.8 Hz, H-6'), 4.66 (1H, *d*, $J = 7.9$ Hz, H-1'' or 1'), 3.95 (1H, *dd*, $J = 12$, 2 Hz) and 3.75 (1H, *dd*, $J = 12$, 5.5 Hz, H-6''), 7.34–7.49 (5H, *m*, H-5, 6, 7, 8 and 9), 3.32–3.41 (5H, *m*, H-4', 2'' or 2', 3'' or 3', 4'' and 5''); ^{13}C NMR (CD_3OD): see Table 1; FABMS *m/z*: 513 $[M+Na]^+$; CD (EtOH; $c\,4.08 \times 10^{-4}$) $\Delta\varepsilon^{19}$: 2.08 (218) (pos. max).

(2RS)-6-O-[2-(4-*hydroxyphenyl*)*propionyl*]-D-*Glucose* (**12**). Amorphous solid: $[\alpha]_D^{24}$ 50.8° (MeOH; *c* 0.24); IR ν_{max}^{KBr} cm^{-1}: 3405, 1720, 1515; ^{1}H NMR (CD_3OD): *δ* 3.77 (1H, *q*, $J = 7$ Hz, H-2), 1.515 (1H) and 1.510, 2H, *d*, $J = 7$ Hz, H-3), 5.14 and 5.11 (each 0.25H, *d*, $J = 3.8$ Hz) and 4.55 and 4.53 (each 0.25H, *d*, $J = 8$ Hz, H-1'), 3.19 (0.5H, *dd*, $J = 9$, 8 Hz, H-2'), 3.74 and 3.72 (each 0.25H, *t*, $J = 9$ Hz, H-3'), 4.02 and 3.98 (each 0.25H, *ddd*, $J = 10$, 5, 2 Hz) and 3.54 and 3.53 (each 0.25H, *ddd*, $J = 10$, 6, 2 Hz, H-5'), 4.46 (0.5H, *dd*, $J = 12$, 2 Hz), 4.22 (0.5H, *dd*, $J = 11.5$, 2 Hz), 4.29 (0.5H, *dd*, $J = 12$, 5 Hz) and 4.28 (0.5H, *dd*, $J = 11.5$, 6 Hz, H-6'), 7.17–7.23 (2H, *m*, H-5 and 9), 6.78–6.84 (2H, *m*, H-6 and 8), 3.28–3.50 (2H, *m*, H-2', 3' and 4'); ^{13}C NMR (CD_3OD): see Table 1; FABMS *m/z*: 351 $[M+Na]^+$.

(2RS)-*β*-D-*Fructofuranosyl* 6-O-[2-(4-*hydroxyphenyl*)*propionyl*]-α-D-*glucopyranoside* (**13**). Amorphous solid: $[\alpha]_D^{19}$ 12.8° (MeOH; *c* 0.36); IR ν_{max}^{KBr} cm^{-1}: 3420, 1720, 1515; ^{1}H NMR (CD_3OD): *δ* 1.52 and 1.51 (each 1.5H, *d*, $J = 7$ Hz, H-3), 5.44 and 5.39 (each 0.5H, *d*, $J = 3.8$ Hz, H-1'), 3.45 and 3.35 (each 0.5H, *dd*, $J = 10$, 3.8 Hz, H-2'), 3.34 and 3.32 (each 0.5H, *dd*, $J = 10$, 9 Hz, H-4'), 4.56 and 4.46 (each 0.5H, *dd*, $J = 12$, 2 Hz) and 4.30 and 4.20 (each 0.5H, *dd*, $J = 12$, 4.5 Hz, H-6'), 4.19 and 4.18 (each 0.5H, *d*, $J = 8$ Hz, H-3'), 7.19–7.25 (2H, *m*, H-5 and 9), 6.79–6.85 (2H, *m*, H-6 and 8), 4.02–4.11 (2H, *m*, H-5' and 4''), 3.63–3.90 (6H, *m*, H-2, 3', 1'', 5'' and 6''); ^{13}C NMR (CD_3OD): see Table 1; FABMS *m/z*: 513 $[M+Na]^+$.

(2RS)-*Ethyl* 2-[4-O-(6-O-*β*-D-*glucopyranosyl*-*β*-D-*glucopyranosyl*)*phenyl*]*propionate* (**14**). Amorphous solid: $[\alpha]_D^{19}$ −62.8° (MeOH; *c* 1.08); IR ν_{max}^{KBr} cm^{-1}: 3430, 1730, 1510; ^{1}H NMR (CD_3OD): *δ* 1.52 (3H, *d*, $J = 7$ Hz, H-3), 4.980 and 4.985 (each 0.5H, *d*, $J = 8$ Hz, H-1'), 4.25 (1H, *dd*, $J = 11.5$, 1.8 Hz) and 3.91 (1H, *dd*, $J = 11.5$, 6 Hz, H-6'), 4.470 and 4.475 (each 0.5H, *d*, $J = 7.6$ Hz, H-1''), 3.94 (1H, *dd*, $J = 12$, 2.2 Hz) and 3.73 (1H, *dd*, $J = 12$, 5.8 Hz, H-6''), 4.19 and 4.18 (each 0.5H, *q*, $J = 7$ Hz, OCH_2), 1.27 (3H, *t*, $J = 7$ Hz, Me), 7.30–7.33 (2H, *m*, H-5 and 9), 7.15–7.19 (2H, *m*, H-6 and 8), 3.75–3.82 (2H, *m*, H-2 and OCH_2), 3.48–3.59, 3H (*m*, H-2', 4' and 5'), 3.27–3.46 (5H, *m*, H-3', 2'', 3'', 4'' and 5''); ^{13}C NMR (CD_3OD): see Table 1; FABMS *m/z*: 541 $[M+Na]^+$.

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