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GLYCOSYLATION OF 2-PHENYLPROPIONIC ACID AND ITS ETHYL ESTER IN SUSPENSION CULTURES OF *NICOTIANA TABACUM*, *DIOSCOREOPHYLLUM CUMMINSII* AND *ACONITUM JAPONICUM**

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Key Word Index—*Nicotiana tabacum*; *Dioscoreophyllum cumminsii*; *Aconitum japonicum*; cell suspension culture; biotransformation; glycosylation; 2-(*RS*)-phenylpropionic acid; glucosyl ester; gentiobiosyl ester; ethyl 6-*O*-[2-(*RS*)-phenylpropionyl]-β-D-glucopyranoside.

Abstract—Suspension cultures of *Nicotiana tabacum* and *Dioscoreophyllum cumminsii* converted 2-(*RS*)-phenylpropionic acid and its ethyl ester into 2-(*RS*)-phenylpropionyl β-D-glucopyranoside, 2-(*RS*)-phenylpropionyl 6-*O*-β-D-glucopyranosyl-β-D-glucopyranoside and 6-*O*-β-D-glucopyranosyl-2-*O*-[2-(*RS*)-phenylpropionyl]-D-glucose which accumulated in the cells. A suspension culture of *Aconitum japonicum* converted these substrates into ethyl 6-*O*-[2-(*RS*)-phenylpropionyl]-β-D-glucopyranoside which was mostly excreted into the medium. The diastereomeric mixture of the glucosyl esters of 2-(*RS*)-phenylpropionic acid was resolved by HPLC to show the ratio of *R*:*S* was 1:1.

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 [1–12] and there has been intensive study of the metabolism of cardenolides by cell cultures of *Digitalis lanata* and other plants [4, 5]. After administration of digitoxin several different reactions occur, including glucosylation, acetylation and hydroxylation. The most important reaction found is the 12β-hydroxylation to convert digitoxin to digoxin which is mostly used is cardiac therapy today. Moreover, it is also well known that suspension cultures of *Catharanthus roseus* can produce the indole alkaloid ajimalicine from the distant precursors tryptamine and secologanin [6]. The biotransformations are mostly stereospecific and generally they involve hydroxylation, acetylation or glycosylation.

The present paper reports a study on the biotransformation of phenylpropanoids. Numerous phenylpropanoids and closely related compounds which are both natural or synthetic are available for human use. 2-Phenylpropionic acid (PPA) and its ethyl ester (PPA-E) are used as important synthetic materials to prepare medicaments and agrichemicals. To obtain compounds pharmacologically more active and difficult to synthesize chemically, 2-(*RS*)-phenylpropionic acid and its ethyl ester as analogues of phenylpropanoids were first administered to plant cell cultures (*Nicotiana tabacum*, *Dioscoreophyllum cumminsii* and *Aconitum japonicum*) and the biotransformation products were investigated.

RESULTS AND DISCUSSION

Conversion products **1** and **2** were isolated by silica gel chromatography and HPLC as colourless needles from methanol extracts of the cultured cells of *N. tabacum* and *D. cumminsii* previously administered PPA. In the ^{13}C NMR spectrum of **1**, the signals assigned to the glucose moiety were observed in addition to those due to the 2-phenylpropionyl group (Table 1). Glucose in **1** is acylated with PPA at the anomeric position as shown by observations of the signal of the anomeric carbon at δ96.3. The ^{13}C NMR spectrum of **2** was similar to that of **1**, and thus the structure of **2** is similar to **1**. In the ^{1}H NMR spectra of **1** and **2**, the signals at δ5.46 and 5.47 (**1** and **2**, respectively) assigned to anomeric protons of glucose were observed with large downfield shifts, suggesting the acylation of glucose at C-1. The large coupling constants (J = 8 Hz) of the anomeric protons in **1** and **2** suggest the configurations, of the anomeric centers in **1** and **2** to be β. The ^{1}H NMR and ^{13}C NMR spectra of **1** were very similar to those of **2** and the differences in chemical shift values between **1** and **2** were less than 0.01 ppm in the ^{1}H NMR and 0.1 ppm in the ^{13}C NMR spectra. Differences in chemical shift values for H-3 of the 2-phenylpropionyl group and for H-6 of the glucose moiety slightly exceeded those in the ^{1}H NMR spectrum. These differences possibly arise from the configuration at C-2 of the 2-phenylpropionyl group. To identify the sugars in **1** and **2**, acid hydrolyses of **1** and **2** with 1N sulphuric acid were carried out to give PPA and glucose. The configurations at C-2 of the 2-phenylpropionyl groups of **1** and **2** were determined by specific rotations and CD spectra. The specific rotation of **1** was −15.0° and that of **2** was +34.0°; the value for a mixture of **1** and **2** (ratio; 1:1) was +6.4°. The CD spectrum of **1** showed a negative maximum at −0.21 and that of **2** gave a positive maximum at +0.66. The CD spectrum of **2** exhibited the same Cotton effect with (2*S*)-phenylpropionic acid and that of **1**, the

*Part 50 in the series 'Studies on Plant Tissue Culture'. For Part 49, see Furuya T., Yoshikawa T., Kimura T. and Kaneko H. (1987) *Phytochemistry* (in press).

Table 1. ^{13}C NMR chemical shifts* and assignments of conversion products 1–5

C	1	2	3	4		5	
1	175.3	175.3	175.4	176.4	175.8	176.5	
2	46.9	46.9	46.9	47.1	46.8	47.0	
3	19.5	19.7	19.6	19.6	19.9	19.2	19.4
4	141.8	141.9	141.8	142.6	142.3	142.3	
5,9	129.9[a]	130.0[b]	129.9[c]	129.8[d]	129.9[e]	129.9[g]	130.0[g]
6,8	129.0[a]	129.0[b]	129.0[c]	129.0[d]	128.9[e]	128.9[g]	
7	128.5	128.5	128.5	128.4	128.2	128.4	
Glc-1	96.6	96.4	96.3	91.4	96.6	104.2	104.3
2	74.2	74.3	74.1	75.8	76.9	75.5	
3	78.4	78.3	78.2	72.2	76.2[f]	78.2	
4	71.3	71.2	71.1	72.1	71.9	72.0	
5	79.2	79.2	78.2	72.2	77.3[f]	75.2	
6	62.6	62.5	69.7	70.2		65.3	65.4
Glc-1′			104.8	105.0			
2′			75.4	75.4			
3′			78.2	78.2			
4′			71.8	71.8			
5′			78.3	78.2			
6′			63.0	63.0			
OCH_2						66.5	
Me						15.8	

*Chemical shift in ppm downfield from TMS, solvent CD_3OD.
[a–g]Column assignments are interchangable.

For 4 and 5, there are two columns for each carbon because 4 is a mixture of α- and β-anomers of the inner glucose, and 5 is a mixture of diastereoisomers of the 2-phenylpropionyl groups at C-2.

Table 2. ^1H NMR chemical shifts* and assignments of conversion products 1–5

H	1	2	3
2	3.82 (1H, *q*, *J* = 7 Hz)	3.82 (1H, *q*, *J* = 7 Hz)	3.82 (1H, *q*, *J* = 7 Hz)
3	1.50 (3H, *d*, *J* = 7 Hz)	1.47 (3H, *d*, *J* = 7 Hz)	1.50 (3H, *d*, *J* = 7 Hz)
5,9 6,8	7.28–7.34 (4H, *m*)	7.28–7.32 (4H, *m*)	7.28–7.34 (4H, *m*)
7	7.21–7.25 (1H, *m*)	7.20–7.26 (1H, *m*)	7.21–7.25 (1H, *m*)
Glc-1	5.46 (1H, *d*, *J* = 8 Hz)	5.47 (1H, *d*, *J* = 8 Hz)	5.44 (1H, *d*, *J* = 8 Hz)
2	3.28 (1H, *t*, *J* = 8 Hz)	‡	§
3	†	‡	3.37 (1H, *dd*, *J* = 8.5, 8.5 Hz)
4	†	‡	3.42 (1H, *dd*, *J* = 9.5, 8.5 Hz)
5	†	‡	3.53 (1H, *ddd*, *J* = 9.5, 5.2 Hz)
6	3.83 (1H, *dd*, *J* = 11.5, 2 Hz)	3.77 (1H, *dd*, *J* = 12, 1.8 Hz)	4.14 (1H, *dd*, *J* = 11.5, 2 Hz)
	3.68 (1H, *dd*, *J* = 11.5, 4.5 Hz)	3.62 (1H, *dd*, *J* = 12, 4.5 Hz)	3.77 (1H, *dd*, *J* = 11.5, 5 Hz)
Glc-1′			4.33 (1H, *d*, *J* = 7.6 Hz)
2′			3.20 (1H, *dd*, *J* = 8.5, 7.6 Hz)
3′			3.35 (1H, *dd*, *J* = 8.5, 8.5 Hz)[a]
4′			§
5′			3.23 (1H, *ddd*, *J* = 10, 5.5, 2.2 Hz)
6′			3.85 (1H, *dd*, *J* = 12, 2.2 Hz)
			3.66 (1H, *dd*, *J* = 12, 5.5 Hz)
OCH_2			
CH_3			

*Chemical shift in ppm downfield from TMS, solvent CD_3OD.
†3.33–3.40 (3H, *m*), ‡3.20–3.45 (4H, *m*), §3.27–3.33 (2H, *m*), ‖3.80–3.90 (2.6H, *m*),
¶3.24–3.30 (2H, *m*), **3.38–3.51 (1.8H, *m*), ††7.23–7.27, 7.28–7.32 (total 5H, *m*), ‡‡3.47–3.52 (2H, *m*).
[a]Column assignments are interchangable.

reverse, indicating the configuration at C-2 of the 2-phenylpropionyl group in **1** to be *R* and that in **2** to be *S*. The Cotton effects of **1** and **2** also agreed with those in the CD spectra of carboxylic acids in ref. [13]. From these results, compound **1** appears to be (2*R*)-phenylpropionyl *β*-D-glucopyranoside and **2** is (2*S*)-phenylpropionyl *β*-D-glucopyranoside.

Conversion products **3** and **4** were isolated as amorphous solids from the methanol extract of the cultured cells of *D. cumminsii* administered PPA, by an isolation method similar to that of **1** and **2**. Acid hydrolysis of **3** with 1N sulphuric acid gave PPA and glucose, as in the case of **1** and **2**. However, the FD mass spectrum of **3** showed a peak at *m/z* 497 $[M+Na]^+$ suggesting the composition of **3** to be one molecule of PPA and two molecules of glucose. A comparison of the ^{13}C NMR spectrum of **3** with those of **1** and **2** revealed an additional set of signals due to a terminal *β*-glucosyl unit in the spectrum of **3**. Glycosylation shifts of the glucose carbons for **1** and **2** were recognized at C-6 (about −7.0 ppm) and C-5 (about +1.0 ppm) as compared with that of **3**. Thus the terminal glucose in **3** is linked to the C-6 position of the inner glucose. The ^1H NMR spectrum of **3** also showed the gentiobiosyl ester of PPA, but the signals of the methyl group at C-3 (at H-3 of the 2-phenylpropionyl group) were observed as two peaks (*δ* 1.47 and 1.50). This indicates that **3** may be a mixture of the gentiobiosyl esters of *R* and *S*-PPA, with a ratio of 1:4 or 4:1 according to the integrated intensity of the methyl protons. Attempts to separate **3** into two diastereoisomers was not successful. On the basis of these chemical and spectroscopic data, **3** was concluded to be a mixture of (2*RS*)-phenylpropionyl 6-*O*-*β*-D-glucopyranosyl-*β*-D-glucopyranoside.

Acid hydrolysis of **4** with 1 N sulphuric acid also gave PPA and glucose. The FD mass spectrum of **4** showed a peak at *m/z* 497 $[M+Na]^+$, suggesting the composition of **4** to be the same as that of **3**. The ^1H NMR spectrum of **4**, measured in methanol at room temperature, exhibited signals showing the presence of the *α*-and *β*-anomers of gentiobiose. The ratio of *α*- and *β*-anomers was judged to be about 3:2 from the integrated intensity of the respective anomeric protons (Table 2). From the ^1H–^1H-2D-NMR spectrum of **4**, the H-2 signal of glucose was coupled to the anomeric proton signals and shifted downfield (*δ* 4.59, *dd*, *J* = 10, 3.5 Hz and 4.70, *dd*, *J* = 9.5, 8 Hz) as compared with that of **3**, indicating the 2-phenylpropionyl group to be attached to the C-2 position. The ^{13}C NMR spectrum of **4** showed signals at *δ* 91.4 and 96.6, corresponding to the anomeric carbons and suggesting the presence of *α*-and *β*-anomers. The ^1H NMR spectrum indicated that **4** may be a mixture of the two diastereoisomers of (*RS*)-PPA, as in the case for **3**, but these diastereoisomers have not been separated.

Conversion product **5** was isolated as an amorphous solid from the ethyl acetate extract of the medium of *A. japonicum* administered PPA-E, by silica gel chromatography and HPLC. Acid hydrolysis of **5** with 1N sulphuric acid gave PPA and glucose with results similar to those of **1** and **2**. The FAB mass spectrum of **5** showed a peak at *m/z* 341 $[MH]^+$ which was larger by 28 mass units than **1**. The ^1H NMR and ^{13}C NMR spectra suggested **5** to be a mixture of diastereoisomers (Tables 1 and 2). In the ^1H NMR spectrum of **5**, a set of the signals due to the ethyl group appeared and those at H-6 of the glucose moiety shifted to a lower field as compared with those of **1**, as shown in Table 2. The ^{13}C NMR spectrum of **5** showed,

4	5
‖	**3,78 (0.5H, *q*, *J* = 7 Hz)** 3.782 (0.5H, *q*, *J* = 7 Hz)
1.47 (1.8H, *d*, *J* = 7 Hz) 1.46 (1.2H, *d*, *J* = 7 Hz)	1.506 (1.5H, *q*, *J* = 7 Hz) 1.504 (1.5H, *d*, *J* = 7 Hz)
7.25–7.35 (4H, *m*)	††
	7.18–7.24 (1H, *m*) ††
	††
5.13 (0.6H, *d*, *J* = 3.5 Hz) 4.80 (0.4H, *d*, *J* = 8 Hz)	4.19 (0.5H, *d*, *J* = 8 Hz) 4.185 (0.5H, *d*, *J* = 8 Hz)
4.59 (0.6H, *dd*, *J* = 10, 3.5 Hz) 4.70 (0.4H, *dd*, J = 9.5, 8 Hz)	3.29 (1*H*, *dd*, *J* = 9, 8 Hz)
‖ **	‡‡
**	3.26 (1H, *dd*, *J* = 9.5, 9 Hz)
3.93 (0.6H, *ddd*, *J* = 10, 5.5, 2 Hz) **	3.37–3.42 (1H, *m*)
4.09 (0.6H, *dd*, *J* = 11.5, 2 Hz) 4.15 (0.4H, *dd*, *J* = 11.5, 2 Hz)	4.39 (0.5*H*, *dd*, *J* = 12, 5 Hz) 4.37 (0.5H, *dd*, J = 12, 5 Hz)
3.76 (0.6H, *dd*, *J* = 11.5, 5.5 Hz) 3.72 (0.4H, *dd*, *J* = 11.5, 5.5 Hz)	4.315 (0.5*H*, *dd*, *J* = 12, 2.2 Hz) 4.31 (0.5H, *dd*, J = 12, 2.2 Hz)
4.31 (0.6H, *d*, *J* = 8 Hz) 4.32 (0.4H, *d*, *J* = 8 Hz)	
3.19 (0.6H, *d*, *J* = 8.5, 8 Hz) 3.18 (0.4H, *d*, *J* = 8.5, 8 Hz)	
3.34 (1H, *dd*, *J* = 9, 8.5 Hz)	
¶	
¶	
3.64 (0.6H, *dd*, *J* = 12, 5 Hz) 3.65 (0.4H, *dd*, *J* = 12, 5 Hz)	
‖	
3.82 (0.5H, *q*, *J* = 7 Hz) 3.815 (0.5H, *q*, *J* = 7 Hz)	
	1.225 (1.5H, *t*, *J* = 7 Hz) 1.22 (1.5H, *t*, *J* = 7 Hz)

in addition to signals due to glucose and the 2-phenylpropionyl group, the following carbon signals: a methyl (δ15.8) and a methylene carrying an oxygen function (66.5). Comparing the ^{13}C NMR spectra of **1** and **5**, acylation shifts of glucose carbons for **5** were observed at C-5 (about -4 ppm) and C-6 (about $+2.8$ ppm). These results indicated that the ethyl and the 2-phenylpropionyl groups are attached to the C-1 and C-6 positions of glucose, respectively. Therefore, **5** is mixture of ethyl 6-*O*-[(2*R*)-phenylpropionyl]-β-D-glucopyranoside and ethyl 6-*O*-[(2*S*)-phenylpropionyl]-β-D-glucopyranoside.

Next, to investigate the conversion ability of each plant and the utilization of the two substrates (the free acid and ester forms) time course experiments were carried out. Each suspension culture, grown for two–three weeks, was administered PPA or PPA-E and was harvested at one–seven days latter. The medium and cells were separately extracted by the methods described in Experimental. The media and methanol extract of the cells were extracted with *n*-butanol saturated with water and then the conversion ratios from the substrates were determined by HPLC.

As shown in Fig. 1, the suspension culture of *N. tabacum* produced four major conversion products **1**, **2**, **3** and **4** which accumulated in the cells and could not be detected in the medium. When PPA was administered, conversion into glycosides **1**, **2** and **3** was observed one day after the administration. Conversion into **1** and **2** already reached about 28% of the maximum conversion ratio at day 1 and then decreased gradually. Conversion into **3** was low at day 1, but increased gradually over a period of seven days. The conversion ratio attained about 7% at seven days. The conversion from PPA-E into **1** and **2** was observed at day 1, but the conversion ratio was about 6% lower than that with the administration of PPA. The conversion ratio then became maximum (about 16%) and remained constant for seven days. Thus one–two days are required for the removal of ethyl group from PPA-E by an esterase. Finally, the conversion ratio from PPA-E became essentially the same as that from PPA. The conversion from PPA-E into **3** was observed at day 3 and then increased gradually to day 7; the conversion ratio reached about 5%. The conversion from PPA or PPA-E into **4** was observed following the production of **3** but the conversion ratio was low.

The suspension culture of *D. cumminsii* (Fig. 2) also produced **1**, **2**, **3** and **4** and two additional unknown products in the cells. The conversion from PPA into **1** and **2** was observed at day 1, but the conversion ratio, about 7%, was lower than that of *N. tabacum*. Thereafter, the ratio remained constant until day 7. Conversion into **3** was observed at day 2 and continued to increase until day 7,

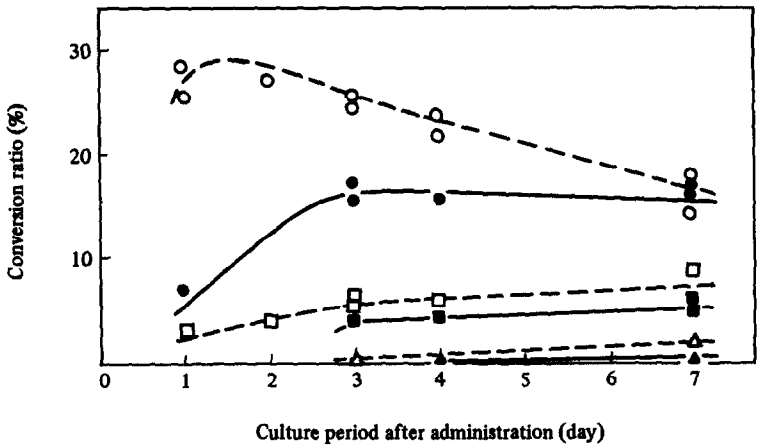

Fig. 1. Time course of the conversion products from PPA or PPA-E: in the cells of *N. tabacum*: O---O, 1 + 2 from PPA; □---□, 3 from PPA; △--△, 4 from PPA; ●——●, 1 + 2 from PPA-E; ■——■, 3 from PPA-E; ▲——▲, 4 from PPA-E.

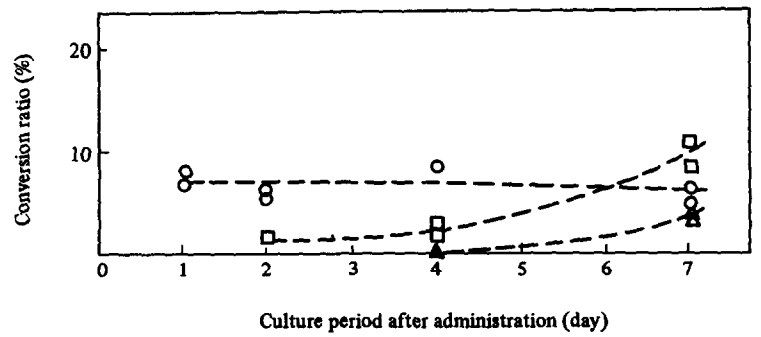

Fig. 2. Time course of the conversion products from PPA, in the cells of *D. cumminsii*: O---O, 1 + 2; □---□, 3; △--△, 4.

with the conversion ratio reaching about 10% at day 7. The conversion from PPA-E into **1** and **2** was observed at a very low level. Although the conversion ratio became almost the same as that from PPA at day 7, the conversion products were only **1** and **2**. A large amount of PPA-E and PPA, administered as substrate into *D. cumminsii*, was observed in the cells, but could not be detected in the suspension cultures of *N. tabacum* and *A. japonicum*. These results show that the suspension culture of *D. cumminsii* has low deethylation and glycosylation ability compared to *N. tabacum* and *A. japonicum*, and **3** may be produced by attachment of glucose onto glucose in **1** and **2**. Compound **4** may be also produced from **3** by acylmigration, as shown in Fig. 3.

As shown in Fig. 4, the suspension culture of *A. japonicum* produced the conversion product **5**, which differed with the products of *N. tabacum* and *D. cumminsii*, most of it was excreted into the medium. Compound **5** was observed at day 1 in the conversion from PPA and at day 2 in that from PPA-E and then the conversion ratio increased to reach about 16 and 21% after day 3 from PPA and PPA-E, respectively. Compound **5** was produced from both PPA and PPA-E thus indicating that the ethyl group of **5** may possibly come from the ethanol which dissolves the substrates, but not from the ethyl group of PPA-E.

Although the suspension cultures of the three plants used in this experiment had high potentiality to form the glycosides of PPA when PPA or PPA-E was administered, they could not stereoselectively form the glycosides

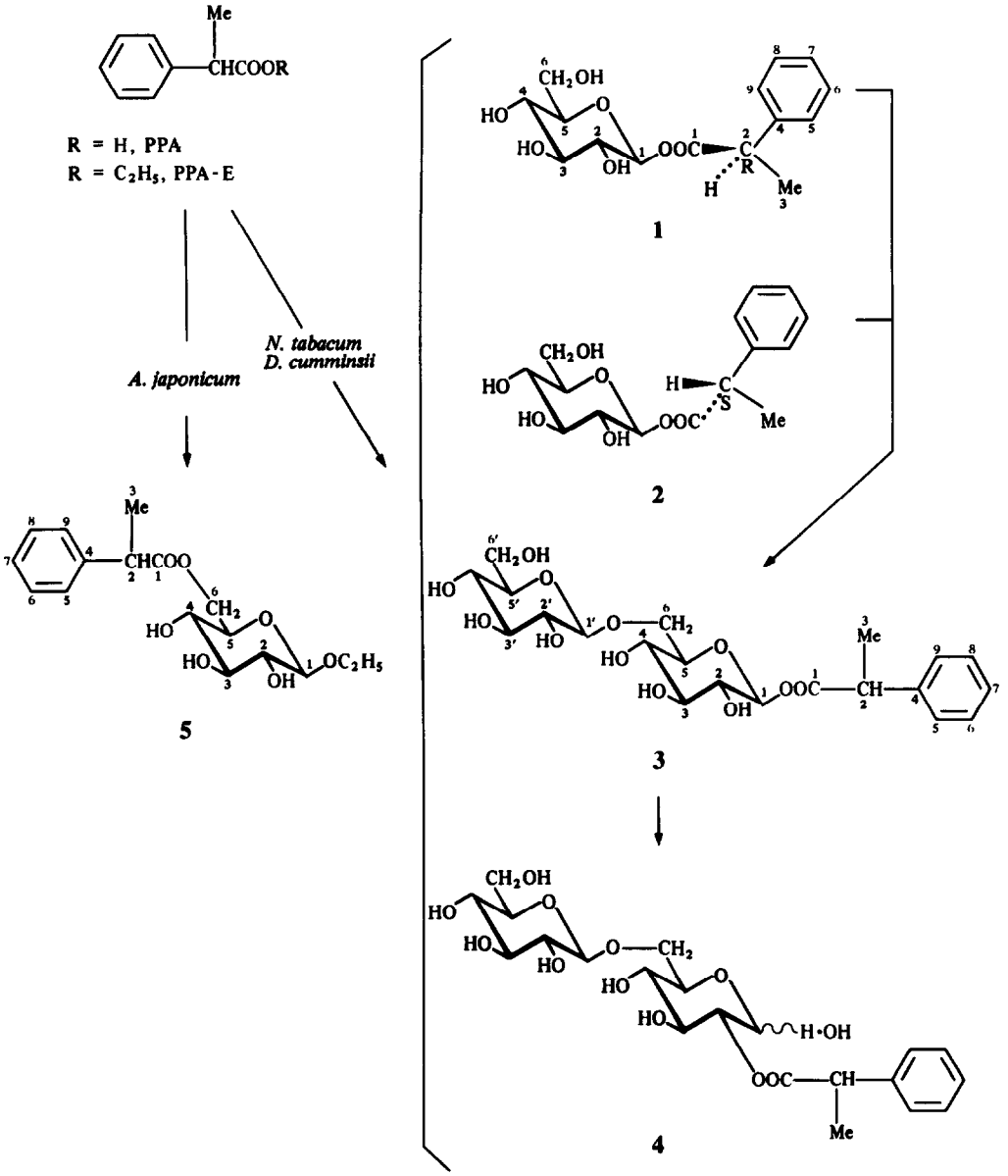

Fig. 3. Possible scheme for the biotransformation of PPA and PPA-E by cell cultures of *N. tabacum*, *D. cumminsii* and *A. japonicum*.

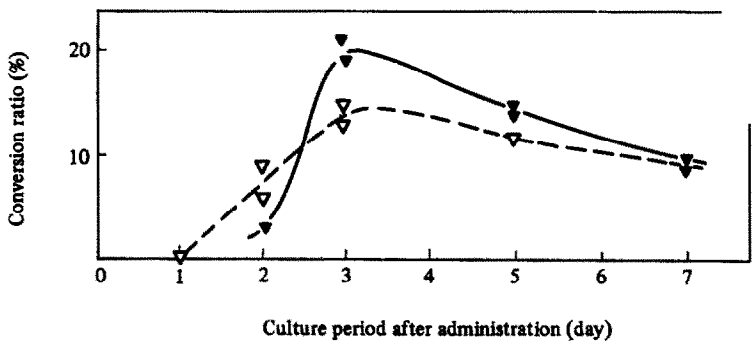

Fig. 4. Time course of the conversion into products 5 from PPA or PPA-E, in the medium of *A. japonicum*: ▽---▽, from PPA; ▼——▼, from PPA-E

of either (*R*)-PPA(PPA-E) or (*S*)-PPA(PPA-E). It is considered that these glycosylation reactions may be mechanisms to decrease the toxity of the substrates and sugars binding to the substrates and binding points of sugars depend primarily on the plant species. However, the diastereoisomer of 2-(*RS*)-phenylpropionyl β-D-gluco: pyranoside among the conversion products could be resolved by HPLC as a result of glycosylation. In *N. tabacum* and *D. cumminsii*, the conversion products accumulated in the cells, whereas in the case of *A. japonicum*, the conversion product was excreted to the medium. Whether this depends on the character of the plant cell or type of the compound is a point requiring further investigation of various substrates and plant cells.

EXPERIMENTAL

Mps: uncorr. NMR: 400 MHz (CD_3OD). FD and FAB MS were taken with a JEOL JMS D-300 and DX-300 instruments equipped with a direct inlet system.

Culture methods. The cell culture of *Nicotiana tabacum* was initiated from the stem of 'Bright Yellow' in 1966 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 [2]. The cell culture of *Aconitum japonicum* was initiated from root grown in Sado, Japan in 1978 and subcultured on the same medium as that used in tobacco cells. Thereafter, the callus was transferred in 1980 to the Revised Tobacco [14] medium containing NAA 1 ppm, kinetin 0.1 ppm and 0.1% Casamino acid, and subcultured on the same medium for 3 weeks. The cell cullture of *Dioscoreophyllum cumminsii* was initiated in 1976 from a stem on the Murashige and Skoog's agar medium containing IAA 1 ppm and kinetin 0.1 ppm, and subcultured on the Revised Tobacco medium containing 2,4-D 1 ppm, kinetin 0.1 ppm and 0.1% Casamino acid for 3 weeks[15].

The calli were transferred to a liquid medium of the same composition in the subculture, and cultured on a rotary shaker at 145 rpm and 25° in the dark. After 2–3 weeks, each 25 mg substrate, after being dissolved in 2 ml of 50% EtOH, was added to 250 ml of each suspension culture and cultured for 1–7 days. The substrates used in this experiment were 2-(*RS*)-PPA (colourless liquid, bp 264–265°) or 2-(*RS*)-PPA-E (colourless liquid, bp 230°) supplied from Nissan Chemical Industries Ltd.

Isolation of conversion products **1** *and* **2**. The suspension culture of *N. tabacum* was separated into the medium and the cells by Nylon cloth, and the latter were homogenized with MeOH. The homogenate was filtered and the residue extracted with MeOH under reflux for 3 hr and filtered. Both filtrates were combined and concentrated, followed by suspension 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 $CHCl_3$–MeOH (9:1) as the eluent. The fraction containing **1** and **2** was collected and a mixture of products **1** and **2** was crystallized from *n*-hexane–EtOH. Products **1** and **2** were separated by HPLC using a Unisil Q C18 column and MeOH–THF–H_2O (15:5:80) as the eluent.

Separation of conversion products **3** and **4**. The *n*-BuOH fraction prepared from the suspension culture of *D. cumminsii* previously administered PPA by the same method as **1** and **2**, 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 fractions containing **1** and **2**, and **3** and **4** were collected. Compounds **3** and **4** were separated by rechromatography on a silica gel column using $CHCl_3$–MeOH (3:1) as the eluent, followed by HPLC using a Unisil Q C18 column and MeOH–THF–H_2O (8:12:80) as the eluent.

Separation of conversion product **5**. The suspension culture of *A. japonicum* was separated into the medium and cells, and the medium was extracted with EtOAc. The EtOAc extract was chromatographed on a column of silica gel using $CHCl_3$–MeOH (16:1) as the eluent. Compound **5** was separated from the eluate by HPLC using a Unisil Q C18 column and MeOH–H_2O (1:1) as the eluent.

Quantitative analysis of conversion products. The media and MeOH extracts of the cells from each 250 ml suspension culture follo addition of each substrate were extracted × 2 with *n*-BuOH saturated with H_2O. The content of the conversion products containing the *n*-BuOH extracts was determined by HPLC. HPLC was carried out using a Unisil Q C18 column (300 × 7.6 mm) to determine the conversion ratio of each product, monitoring with a differential refractometer and UV absorption at 254 nm. The eluents were MeOH–H_2O–AcOH (38:60:2) and MeOH–H_2O (1:1), and the flow rate, 2.0 ml/min. R_t(min) were as follows:

	1	2	3	4	PPA	5	PPA-E
MeOH–H_2O–AcOH (38:60:2)	15.1	15.2	10.8	12.1	39.2	–	–
MeOH–H_2O (1:1)	–	–	–	–	19.6	21.0	50.6

Acid hydrolysis of conversion products. The conversion products were hydrolysed with 1 N H_2SO_4 in a sealed tube to a vacuum system at 70° for 5 hr. The hydrolysate was applied to a column of Diaion HP-20 and eluted with MeOH following elution of sugars with H_2O. From the MeOH eluate, PPA was identified by a direct comparison of TLC behavior (R_f 0.56, C_6H_6–dioxane–AcOH, 90:25:4; R_f 0.53; C_6H_6–MeOH–AcOH, 45:8:4) with that of an authentic sample. The H_2O eluate was neutralized through a column of Amberlite IR 45 (OH^-). After elution with H_2O, the sugars in the eluate were identified with authentic glucose by TLC (R_f 0.30, Me_2CO–H_2O–$CHCl_3$–MeOH, 75:5:10:10; R_f 0.20, $CHCl_3$–MeOH–H_2O, 6:4:1), and the eluate was further reduced with $NaBH_4$ for 2 hr. After neutralization with Amberlite IR 120B (H^+), the soln was evapd and boric acid was removed by repeated addition and evaporation of MeOH. The reduced products were acetylated with Ac_2O–pyridine (1:1) at 80° for 2 hr, and subjected to GC on a glass column (2 m × 3 mm) packed with 3% ECNSS-M on Gas Chrom Q (100–120 mesh), the GC was operated at 195° with a N_2 flow rate of 50 ml per min.

(2R)-*Phenylpropionyl β-*D*-glucopyranoside* (**1**). Colourless needles, mp: 136–137°, $[\alpha]_D^{25}$ −15.0° (EtOH; *c* 1.15); IR ν_{max}^{KBr} cm^{-1}: 3400, 1730; ^1H NMR (CD_3OD): see Table 2; ^{13}C NMR (CD_3OD): see Table 1; FAB MS *m/z*: 313 $[MH]^+$; CD (EtOH; *c* 6.73×10^{-4}) $\Delta\varepsilon^{24}$: −0.21 (222) (neg. max).

(2S)-*phenylpropionyl β-*D*-glucopyranoside* (**2**). Colourless needles, mp: 166–167°, $[\alpha]_D^{25}$ +32.4° (EtOH; *c* 1.36); IR ν_{max}^{KBr} cm^{-1}: 3420, 1755; ^1H NMR (CD_3OD): see Table 2; ^{13}C NMR (CD_3OD): see Table 1; FAB MS *m/z*: 313 $[MH]^+$; CD (EtOH; *c* 5.12×10^{-4}) $\Delta\varepsilon^{24}$: +0.66 (223) (pos. max).

2-*Phenylpropionyl* 6-O-*β-*D*-glucopyranosyl-β-*D*-glucopyranoside* (**3**). Amorphous solid: $[\alpha]_D^{27}$ −25.0° (EtOH; *c* 0.92); UV λ_{max}^{EtOH} nm (log ε): 204 (3.92), 251 (2.24), 257 (2.31), 263 (2.16); IR ν_{max}^{KBr} cm^{-1}: 3405, 1745; ^1H NMR (CD_3OD): see Table 2; ^{13}C NMR (CD_3OD): see Table 1; FD MS *m/z*: 497 $[M + Na]^+$; CD (c = 1.94×10^{-4}, EtOH) $\Delta\varepsilon^{23}$: −1.41 (224) (neg. max).

6-O-*β-*D*-Glucopyranosyl-2-O-(2-phenylpropionyl)-*D*-glucose* (**4**). Amorphous solid: $[\alpha]_D^{27}$ +4.2° (EtOH; *c*0.85); UV λ_{max}^{EtOH} nm (log ε): 204 (1.92), 251 (2.24), 257 (2.31), 263 (2.61); IR ν_{max}^{KBr} cm^{-1}: 3390, 1730; ^1H NMR (CD_3OD): see Table 2; ^{13}C NMR (CD_3OD): see Table 1; FD MS *m/z*: 497 $[M + Na]^+$; CD (EtOH; *c* 1.78×10^{-4}) $\Delta\varepsilon^{23}$: −3.26 (221) (neg. max).

Ethyl 6-O-(2-*phenylpropionyl*)-*β-*D*-glucopyranoside* (**5**). Amorphous solid: $[\alpha]_D^{27}$ −5.9° (EtOH; *c* 3.14), IR ν_{max}^{KBr} cm^{-1}: 3420, 1735; ^1H NMR (CD_3OD): See Table 2; ^{13}C NMR (CD_3OD): see Table 1; FAB MS *m/z*: 341 $[MH]^+$.

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