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PURIFICATION AND PROPERTIES OF UDPAPIOSE: 7-*O*-(β-D-GLUCOSYL)-FLAVONE APIOSYLTRANSFERASE FROM CELL SUSPENSION CULTURES OF PARSLEY

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SUMMARY

1. An apiosyltransferase catalyzing the transfer of the apiosyl moiety of UDP-apiose to 7-*O*-(β-D-glucosyl)-apigenin to form 7-*O*-(β-D-apiofuranosyl(1→2)β-D-glucosyl)-apigenin (apiin) has been isolated from cell suspension cultures of parsley. The enzyme has been purified 123-fold by protamine sulfate and ammonium sulfate precipitation and chromatography on DEAE-cellulose, Sephadex G-100 and hydroxylapatite.

2. The UDP apiosyltransferase is a soluble enzyme with a pH optimum of 7.0. Cofactors are not required.

3. The enzyme is specific for UDPapiose as glycosyl donor. 7-Glucosides of flavones, flavanones and isoflavones, apigenin-7-glucuronide and glucosides of *p*-substituted phenols can function as acceptors. Apigenin-7-*O*-(β-D-glucoside) and biochanin A (5,7-dihydroxy-4′-methoxyisoflavone)-7-glucoside are the best acceptors. No reaction takes place with flavonol-3-glucoside, flavonol-7-glucoside, apigenin-8-C-glucoside, aglycones of flavonoids or free glucose.

INTRODUCTION

In the course of our work on the biosynthesis of apiin (7-*O*-[β-D-apiofuranosyl-(1→2)-β-D-glucosyl]-5,7,4′-trihydroxyflavone) in young parsley plants[1,2] and in cell suspension cultures of this plant[3], we have described briefly an enzyme which catalyzes the transfer of the apiosyl moiety of UDPapiose[4] to 7-*O*-(β-D-glucosyl)-apigenin to form apiin[1]. In cell suspension cultures of parsley the activity of the apiosyltransferase is drastically increased by prior illumination of the cells. Ten days after inoculation maximum enzyme activity is reached about 24 h after onset of illumination[3].

In the present paper we describe the partial purification of the apiosyltransferase from cell cultures of parsley and report some of its properties.

MATERIAL AND METHODS

Substrates and reference compounds

Flavonoids and their glycosides were taken from our laboratory collection. Furcatin was a gift from Professor S. Hattori, Tokyo, and lanceolarin a gift from Professor T. R. Seshadri, New Delhi. *p*-Nitrophenyl-α- and -β-glucosides were purchased from Merck, Darmstadt, and bovine serum albumin from Serva, Heidelberg. UDP-D-[U-^{14}C]glucuronic acid (302 μCi/μmole) was purchased from the Radiochemical Centre Amersham.

Partial hydrolysis of furcatin to p-vinylphenol glucoside

10 mg of furcatin were heated to 95 °C for 30 min with 0.5 ml of 1% trifluoroacetic acid. The acid was removed *in vacuo* and *p*-vinylphenol glucoside purified by chromatography on silica gel plates with a fluorescence indicator (Merck, Darmstadt) with solvent-system 6 (see below). The zone of *p*-vinylphenol glucoside (R_F 0.67) was eluted with water.

7-O-(β-D-glucosyl)-apigenin

Apigenin-7-glucoside was obtained by partial hydrolysis of apiin[5] with 2% trifluoroacetic acid. 2 mg of apiin were heated to 100 °C for 15 min with 0.25 ml of the acid. The acid was removed *in vacuo* and apigenin-7-glucoside was purified by paper chromatography (paper prewashed with methanol, 10% acetic acid and 0,01 M EDTA) with butan-1-ol–acetic acid–water (20:1:4, by vol.); $R_F = 0.49$.

Chromatographic methods

For descending paper chromatography on Schleicher Schüll 2043 b, prewashed with 0.01 M EDTA and water, the following solvent systems were used: (1) 15% acetic acid; (2) butan-1-ol–acetic acid–water (4:1:5, by vol., upper phase); (3) 95% ethanol–1 M ammonium acetate (pH 3.8) (5:2, by vol.); (4) ethylacetate–pyridine–water (8:1:2, by vol.).

Thin-layer chromatography was performed on polyamide–cellulose (15:6, by wt, Macherey and Nagel) with: (5) chloroform–methanol–methylethyl ketone–acetylacetone (66:20:10:2, by vol.) and on silica gel (Merck, Darmstadt) with (6) ethylacetate–methylethyl ketone–formic acid–water (5:3:0,6:1, by vol.).

Preparation of UDP-D-[U-^{14}C]xylose and UDP-L-[U-^{14}C]arabinose

The incubation mixture from the UDPapiose synthetase reaction (see below) was applied to paper and chromatographed for 5 days at 4 °C with solvent system 3. After this time 5 radioactive zones were detected (R_{UDPG}-values in parentheses): UDPglucuronic acid (0.45); minor unidentified compound (0.64); UDP-L-arabinose (0.88); UDP-D-xylose (1.0) and UDPapiose (1.2). The zones of UDPxylose and UDPapiose were not completely separated. The nucleotides were eluted from paper at 4 °C with water and the eluates lyophilized. After hydrolysis with glacial acetic acid the sugars were identified by chromatography on paper with solvent system 4.

Cell suspension cultures

Cultivation of cell cultures of *Petroselinum hortense* has been described previ-

ously[3]. The enzyme was extracted from cells which had been illuminated for 24 h with white light[3] prior to being harvested 10 days after the cultures were started.

Measurement of radioactivity

Measurements of radioactivity were made with a Beckman model LS-233 scintillation spectrometer. A toluol mixture (5 g 2,5-diphenyloxazole (PPO) in 1 l toluol) or dioxane mixture (5 g PPO and 100 g naphthalene in 1 l dioxane) was used. Counting yield on polyamide was about 55% and on paper about 65%.

Enzyme assay

(a) Preparation of UDP[U-^{14}C]apiose. The incubation mixture consisted of 0.083 nmole (0.025 μCi) UDP[U-^{14}C]glucuronic acid, 0.15 μmole NAD, 2 μmoles Tris–HCl buffer (pH 7.5) and 50 μl of apiose synthetase[4] (about $3 \cdot 10^{-5}$ units) purified through the Sephadex G-200 step in a total volume of 80 μl.

(b) Test for transferase. 50 μl of the above incubation mixture (containing 11 000 to 16 000 dpm UDP[^{14}C]apiose and UDP[^{14}C]xylose, UDP[^{14}C]arabinose and unreacted substrate), 5 μl of a solution of apigenin-7-glucoside (1.2 mg in 1 ml of ethyleneglycolmonomethylether), 0.2 M Tris–HCl (pH 7.0) and enzyme in a total volume of 105 μl were incubated for 15 min at 30 °C. The protein concentration should be such that not more than 25% of the UDPapiose reacts in 15 min.

At the end of this period 10 μl of a solution of apiin (8 mg in 1 ml of ethyleneglycolmonomethylether) were added and the mixture was applied immediately as a 10 cm wide band to the paper and chromatographed for 6 to 15 h with 15% acetic acid. The apiin zone (R_F = 0,35) was detected under ultraviolet-light (350 nm), eluted and counted in the toluol mixture; the UDPsugars run with the front in this solvent system. The background was determined in an incubation without enzyme.

When acceptors were used whose transfer products had an R_F value above 0.6 in 85% acetic acid a polyamide–cellulose thin-layer plate (0.3 mm) with solvent system 5 was used for separation of the products.

Enzyme purification

All operations were carried out at 4 °C. The steps up to and including DEAE-cellulose column chromatography were carried out without delay.

About 530 g (wet wt) of cells were suspended in 265 ml of 0.2 M Tris–HCl (pH 7.5) plus 265 μl mercaptoethanol in a 1 l beaker and homogenized in an ice bath for 2 min with an Ultra Turrax (Janke and Kunkel, type T 45 N, 10 000 rev./min). Homogenization was then stopped for 1 min in order to keep the temperature low. The procedure was repeated for a total homogenization time of 15 min. The homogenate was centrifuged for 10 min at 27 000 $\times$ g.

The supernatant (610 ml) was stirred for 15 min with 33 g Dowex 1-X2 (Cl^- form equilibrated with 0.2 M Tris–HCl, pH 7.5) and the Dowex filtered off through glass wool. To the filtrate was added over a period of 10 min 60 ml of a cold (4 °C) solution of protamine sulfate (filtrate of a 2% solution adjusted to pH 7.5 with sodium hydroxide). The solution was stirred for 10 min and then centrifuged at 27 000 $\times$ g for 10 min.

Protein in the supernatant was precipitated by addition of solid $(NH_4)_2SO_4$ to 100% saturation. The precipitate was collected by centrifugation at 27 000 $\times$ g for

10 min and dissolved in 27 ml $5 \cdot 10^{-2}$ M Tris–HCl of pH 7.5 containing $1 \cdot 10^{-3}$ M dithioerythritol. This solution was chromatographed on a column (3.5 cm × 30 cm) of Sephadex G-25 with the same buffer.

The protein fraction (38 ml) was applied to a DEAE-cellulose column (2 cm × 16 cm, prewashed with 0.5 M NaOH, 0.5 M HCl and water) equilibrated with the above buffer and the column washed with the same buffer. Protein was eluted with a linear gradient of 500 ml $5 \cdot 10^{-2}$ M Tris–HCl, pH 7.5, containing $2 \cdot 10^{-3}$ M dithioerythritol and 500 ml $4 \cdot 10^{-1}$ M Tris–HCl, pH 7.5, containing $2 \cdot 10^{-3}$ M dithioerythritol at a flow rate of 80 ml/h. The enzyme was eluted between 0.22 M to 0.27 M Tris buffer. Fractions with the highest enzymatic activity (approx. 100 ml) were concentrated to 4 ml by filtration through a "Diaflo" concentrator (Amicon, Model 50, ultrafiltration cell).

The concentrated solution was then chromatographed with $2.5 \cdot 10^{-2}$ M sodium phosphate buffer, pH 7.0, containing $2 \cdot 10^{-3}$ M dithioerythritol on a column (2.5 cm × 40 cm) of Sephadex G-100, equilibrated with the same buffer, at a flow rate of 10 ml/h.

Fractions with the highest enzymatic activity (approx. 24 ml) were absorbed on a column (2.5 cm × 2 cm) of hydroxylapatite (Bio Gel HTP, Bio Rad Laboratories, Richmond, Calif.), equilibrated with $2.5 \cdot 10^{-2}$ M sodium phosphate buffer, pH 7.0. The column was washed with the same buffer containing $4 \cdot 10^{-3}$ M dithioerythritol until no more protein was eluted. Enzyme was then eluted with a linear buffer gradient prepared from 50 ml $2.5 \cdot 10^{-2}$ M sodium phosphate buffer, pH 7.0, containing $4 \cdot 10^{-3}$ M dithioerythritol and 50 ml of $1.5 \cdot 10^{-1}$ M of the same buffer at a flow rate of 50 ml/h. The peak of enzyme activity appeared at about $9.5 \cdot 10^{-2}$ M sodium phosphate buffer.

Incubation of [2-^{14}C]apigenin-7-O-glucoside with nucleotide sugars

50 nmoles or 200 nmoles of each nucleotide sugar were incubated with 12 nmoles of [2-^{14}C]apigenin-7-glucoside (26 000 dpm, dissolved in 5 μl ethyleneglycolmonomethylether), 20 μl apiosyltransferase and 0.2 M Tris–HCl (pH 7.0) in a total volume of 100 μl for 60 min at 30 °C. At the end of this period 80 μg of apiin were added and the mixture was chromatographed on a silica gel plate with solvent system 6. The apiin zone ($R_F = 0.53$) was eluted and counted in the toluol mixture.

RESULTS

Enzyme assay

The enzymatic synthesis of apiin from 7-*O*-(β-D-glucosyl)apigenin was determined by assay for the incorporation of radioactivity from UDP[U-^{14}C]apiose. The difficulty was that UDPapiose could not be obtained in pure form and was present in the assay very probably at a much lower concentration than the saturating concentration.

When UDP-D-[U-^{14}C]glucuronic acid is incubated with the partially purified apiose synthetase from cell cultures of parsley[4] or from *Lemna minor*[6] a mixture of ^{14}C-labelled UDPapiose, UDPxylose, UDParabinose (the latter only with parsley enzyme) and unreacted substrate is formed. UDPapiose can be only partially separated from UDPxylose by paper chromatography in solvent system 3 for 1 week

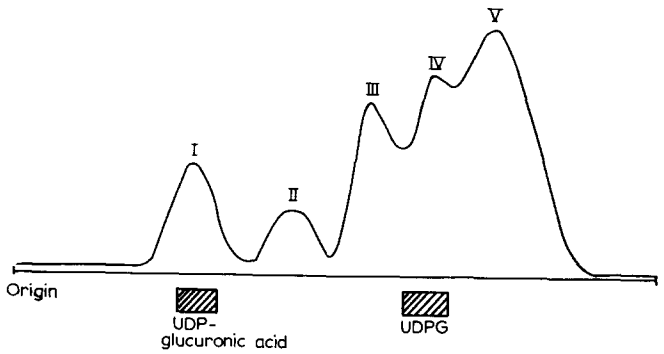

Fig. 1. Paper chromatographic separation of radioactive products from apiose synthetase incubation. I, UDPglucuronic acid; II, unidentified; III, UDP-L-arabinose; IV, UDP-D-xylose; V, UDPapiose.

(Fig. 1). It was not possible to obtain pure UDPapiose by rechromatography of Zone V (Fig. 1) since a strong decomposition of UDPapiose to the cyclic apiose-1,2-phosphate[7] could not be avoided under the conditions tested. The mixture of UDPsugars enzymatically prepared for each transfer test was therefore used in the assay described under Methods. The amount of UDP[U-^{14}C]apiose present in the incubation was determined in each case by hydrolysis of an aliquot of the UDPapiose synthetase incubation[6]. Under the conditions specified the transfer reaction was linear with protein concentration up to 25% conversion (Fig. 2) and time up to 15 min (Fig. 3) from the second purification step (Table I) on.

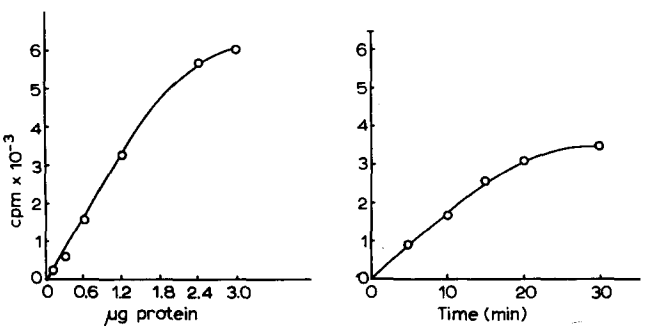

Fig. 2. Dependence of reaction rate on enzyme concentration. UDPapiose, 9000 cpm.
Fig. 3. Dependence of reaction rate on time. UDPapiose, 9000 cpm.

Purification of UDP apiosyltransferase

The purification procedure for the apiosyltransferase is summarized in Table I. A 123-fold purification of the enzyme could be achieved by this procedure. It has already been reported that a partial separation of the apiosyltransferase from the UDPglucose: apigenin-7-*O*-glucosyltransferase[8] is obtained on DEAE-cellulose and that complete separation of the two transferases is achieved on the hydroxylapatite column with a linear gradient of phosphate buffer[8].

In none of the applied separation methods listed in Table I or in analytical disc electrophoresis[8] was more than one peak of apiosyltransferase activity present. The same result was obtained when biochanin A-7-glucoside (see below) instead of api-

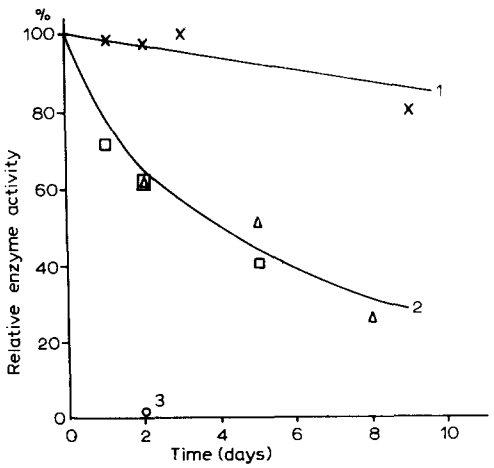

Fig. 4. Enzyme stability under various conditions. For conditions see Table II.

TABLE I

PURIFICATION OF APIOSYLTRANSFERASE

One enzyme unit is defined as the enzyme quantity which catalyzes the formation of 1 μmole apiin at a UDPapiose concentration of 0.03 nM in 1 min at 30 °C in the enzyme assay.

Purification step	*Protein (mg)*	*Specific activity (units × 10^2/mg protein)*	*Purification (-fold)*	*Recovery (%)*
1. Crude extract	1200	—	—	—
2. Dowex supernatant	1200	1.65	1	100
3. Protamine sulfate	610	3.3	2	100
4. Ammonium sulfate and Sephadex G-25	520	3.3	2	85
5. DEAE-cellulose	114	10	6.1	58
6. Sephadex G-100	26	29	17.4	38
7. Hydroxylapatite	4	202	123	35

genin-7-glucoside was used as acceptor. The peak of enzymatic activity for apiose transfer to apigenin-7-glucoside coincided with that for transfer to biochanin A-7-glucoside. It can therefore be stated with confidence that only one apiosyltransferase is present in parsley cell cultures.

Properties of the UDP apiosyltransferase

Stability of the enzyme. The influence of temperature, SH reagents and the type of buffer on enzyme stability is shown in Fig. 4 and in Table II. The enzyme after DEAE or Sephadex G-100 chromatography was most stable at −20 °C in Tris–HCl with addition of dithioerythritol at a protein concentration of 0.5 to 1 mg/ml, whereas a solution of the enzyme after hydroxylapatite chromatography in sodium phosphate buffer (pH 7.0) underwent a rapid loss of activity upon freezing. The enzyme is most stable between pH 6.0 and 8.0. At pH-values below 6.0 and above 8.0 enzyme activity rapidly declines.

pH optimum. The apiosyltransferase has a pH optimum of about 7.0 (Fig. 5).

TABLE II

COMPOSITION OF SOLUTIONS FOR STABILITY TEST OF ENZYME

The solutions were kept at 4 °C for the time indicated on Fig. 4 and the enzyme activity was then determined in the standard assay. DTE, dithioerythritol.

Curve No. (symbol) in Fig. 4	*Last purification step (Table I)*	*Buffer (pH 7.0)*	*Addition*
1 (×—×)	Hydroxylapatite	0.09 M Phosphate	0.004 M DTE
2 (□—□)	DEAE-Cellulose	0.25 M Tris–HCl	0.01 M DTE
(△—△)	Sephadex G-100	0.025 M Phosphate	0.002 M DTE
3 (○)	Sephadex G-200	0.025 M Phosphate	—

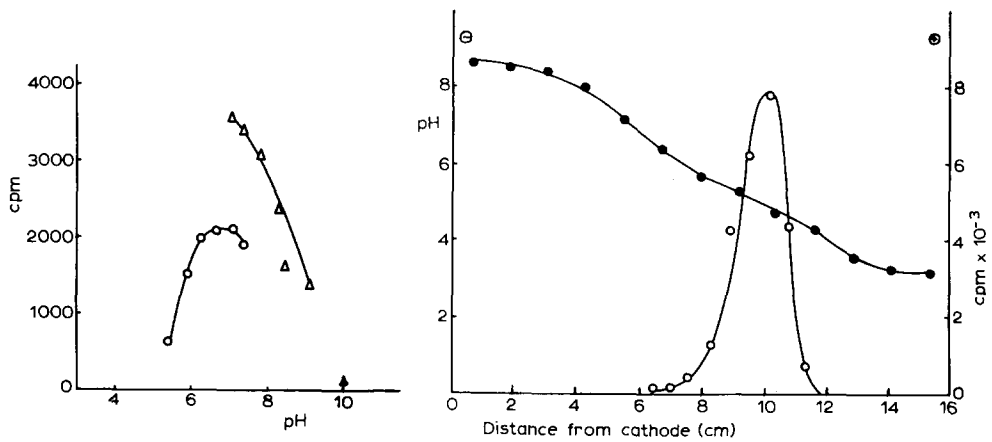

Fig. 5. Dependence of activity on pH. ○—○, 0.4 M phosphate buffer; △—△, 0.4 M Tris–HCl buffer; ▲, 0.4 M Tris–glycine buffer.

Fig. 6. Isoelectric focusing of apiosyltransferase on Sephadex G-75. ○—○, enzyme activity; ●—●, pH.

The yield of apiin is higher in Tris–HCl than in phosphate buffer. The rapid decline of enzyme activity at pH values above 7 could be partially due to the instability of UDPapiose at alkaline pH[7].

Isoelectric point. The isoelectric point of the enzyme was determined to be about 4.8 by isoelectric focusing on a Sephadex G-75 thin-layer plate[8,9] (Fig. 6). When the enzyme was applied to the cathode side of the plate about 60% of enzymatic activity could be recovered after electrofocusing.

Molecular weight. The estimation of the molecular weight of the enzyme on a Sephadex G-100 column has been described previously[8]. The value obtained was about 50 000.

Influence of SH reagents and inorganic ions

Addition of dithioerythritol stimulates enzyme activity up to 35%. The optimal concentration was about $6 \cdot 10^{-3}$ M. *p*-Chloromercuribenzoate at a concentration of 10^{-3} M inhibits the reaction completely and the same concentration of iodoacetamide causes an inhibition of 55%. This inhibition can be completely reversed by cysteine.

EDTA had no influence on the reaction. Bivalent metal ions at a concentration

TABLE III

INFLUENCE OF NUCLEOTIDES AND NUCLEOTIDESUGARS ON ENZYMATIC ACTIVITY

Additions were made to the enzyme assay. Enzyme activity without addition = 100%.

Addition	10^{-2} *M* (%)	10^{-4} *M* (%)	10^{-6} *M* (%)
UMP	0	50	100
UDP	0	25	100
UDPglucuronic acid	–	100	100
UDPG	–	85	100
UTP	0	50	100
ATP	100	100	100

of 10^{-2} M inhibit the reaction in the order $Mn^{2+} > Co^{2+} > Ca^{2+} > Mg^{2+}$, whereas NH_4^+ and K^+ had no influence at this concentration.

Influence of nucleotides, nucleotidesugars, albumin and other possible effectors

The influence of a number of nucleotides and nucleotidesugars on the activity of the apiosyltransferase is shown in Table III. Of the substances tested UDP is the strongest inhibitor of the enzyme. By addition of 100 μg albumin to the incubation a significant stabilization of enzyme activity was achieved.

Apiin, apigenin-7-glucoside and chrysoeriol-7-glucoside inhibited the reaction only above a concentration of 10^{-4} M.

Donor specificity

In the enzyme assay only radioactive apiin could be detected though the incubation mixture also contained UDP[^{14}C]glucuronic acid, UDP[^{14}C]xylose and UDP[^{14}C]arabinose. Furthermore, when an incubation was carried out with a mixture of UDP-D-[^{14}C]xylose (230 000 cpm) and UDP-L-[^{14}C]arabinose (120 000 cpm), which had been isolated from an apiose synthetase incubation, no radioactive transfer product could be detected. In order to test the specificity for other nucleotide sugars, [2-^{14}C]apigenin-7-glucoside[8] was incubated with UDP-D-glucose, UDP-D-galactose, UDP-D-glucuronic acid, UDP-D-xylose, and TDP-D-glucose and the transferase. In none of the incubations was a transfer product detected. On the basis of the sensitivity of the test it is possible to state that these nucleotide sugars are less effective donors than UDPapiose by at least a factor of 10^3. Variation of the base portion of UDP-apiose was not possible since other glucuronic acid nucleotides are not available. UDPglucose dehydrogenase (EC 1.1.1.22) is specific for uracil as base[15].

Acceptor specificity

Qualitative tests showed that flavone-, flavanone- and isoflavone-7-glucosides, apigenin-7-glucuronide and glucosides of *p*-substituted phenols could function as acceptors for apiose transfer, whereas kaempferol-3- and -7-*O*-glucoside and flavonoid aglycones could not function as acceptors. The relative yields of transfer product with different acceptors are listed in Table IV.

In the case of biochanin A-7-glucoside (5,7-dihydroxy-4'-methoxyisoflavone-7-*O*-[β-D-glucoside]), the transfer product had the same R_F values as an authentic

TABLE IV

RELATIVE YIELDS OF TRANSFER PRODUCT WITH DIFFERENT ACCEPTORS

Conditions were those of the enzyme assay (10 000 cpm UDPapiose). Yields are related to the standard assay = 100%.

Acceptor	*Concentration (M)*	*Yield*	K_m*
Apigenin-7-glucoside	$1 \cdot 10^{-4}$	100	$6.6 \cdot 10^{-5}$
Biochanin-A-7-glucoside	$1 \cdot 10^{-4}$	100	
Formononetin-7-glucoside	$5 \cdot 10^{-5}$	66	
	$5 \cdot 10^{-4}$	90	
Chrysoeriol-7-glucoside	$1 \cdot 10^{-4}$	47	
Luteolin-7-glucoside	$5 \cdot 10^{-3}$	36	
Naringenin-7-glucoside	$5 \cdot 10^{-4}$	27	
Apigenin-7-glucuronide	$1 \cdot 10^{-4}$	25	
p-Vinylphenol-glucoside	$5 \cdot 10^{-3}$	25	
p-Nitrophenyl-*α*-glucoside	$1 \cdot 10^{-3}$	<3	
p-Nitrophenyl-*β*-glucoside	$1 \cdot 10^{-4}$	7	
	$1 \cdot 10^{-3}$	22	

* The K_m value was determined according to Lineweaver and Burk at a concentration of UDPapiose (approx $2.7 \cdot 10^{-7}$ M) which was below the saturating concentration.

sample of lanceolarin[10] (biochanin A-7-*O*-[*β*-D-apiofuranosyl(1→2)-*β*-D-glucoside]) on polyamide with solvent system 5 and on paper with solvent systems 1 and 2 and on hydrolysis gave apiose as the only radioactive sugar. The transfer products with the other acceptors were detected only by their different R_F values from those of the acceptors. The comparison between the *α*- and *β*-glucosides of *p*-nitrophenol as acceptors gives an indication that the transferase is specific for *β*-glucosides.

The values in Table IV show that biochanin A-7-glucoside, which does not occur in parsley, is as good an acceptor as apigenin-7-glucoside. Substitution of the B-ring with a 3'-hydroxyl group (luteolin) or 3'-methoxyl group (chrysoeriol) decreases the acceptor ability.

DISCUSSION

Apiin and the corresponding apiosylglucoside of chrysoeriol occur both in parsley[11] and in cell suspension cultures of this plant[12]. Together with the isolation of the UDP glucosyltransferase[8] the results presented in this paper prove that apiin is formed by a stepwise transfer of glucose and apiose from the corresponding UDP-sugars which is catalysed by two different specific transferases.

On the basis of observed changes of enzyme activity with time after illumination of the cultures we had proposed that both transferases are directly involved in the biosynthesis of flavone-apiosylglucosides in parsley[3].

Only one apiosyltransferase could be detected in the cell cultures. The enzyme is highly specific for the glycosyl donor. Only the transfer of the apiosyl moiety of a number of UDPsugars tested was catalysed by the enzyme. The acceptor specificity is not so pronounced (Table IV), but only *β*-glucosides can function as acceptors. Isoflavone-7-*O*-glucosides, which are good acceptors, have not been found in parsley, but an apiofuranosylglucoside of biochanin A (lanceolarin) has been found in *Dahlbergia lanceolaria*[10].

The structure of the disaccharide apiin has been unequivocally established as β-D-apiofuranosyl(1→2)-β-D-glucoside[13,14]. According to chemical evidence apiose is also attached to the 2-position of glucose in the corresponding chrysoeriolglycoside[11] and in lanceolarin[10]. In the case of the apiosylglycoside of *p*-vinylphenol (furcatin) from *Viburnum furcatum*[15], on the other hand, the consumption of 4 moles of periodate in periodate oxidation points to a linkage of apiose to the 6-position of glucose[15]. With *p*-vinylphenol as acceptor a transfer product was obtained with the parsley enzyme which had the same R_F value on polyamide as furcatin. If the nature of the aglycone has no influence on the course of the apiosyltransfer this result could be explained by one of the following assumptions: (1) The apiosyl(1→2)glucoside and apiosyl(1→6)-glucoside are not separated in our chromatographic system; (2) furcatin is in fact the apiosyl(1→2)glucoside of *p*-vinylphenol and not the 1→6 glucoside.

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