Hesperetin 7-*O*-Glucoside and Prunin in *Citrus* Species (*C. aurantium* and *C. paradisi*). A Study of Their Quantitative Distribution in Immature Fruits and as Immediate Precursors of Neohesperidin and Naringin in *C. aurantium*

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The flavanone glucosides prunin (naringenin 7-*O*-glucoside) and hesperetin 7-*O*-glucoside were isolated and identified in the immature fruits of *Citrus aurantium*. Only prunin was identified in *Citrus paradisi*. This is the first study concerned with the isolation of hesperetin 7-*O*-glucoside from *Citrus* tissues and with the quantitative distribution of both flavanone glucosides during the development of *C. aurantium* fruits. Both reach maximum concentration during the first stages of growth, gradually decreasing until the fruits reach maximum development. The total content per fruit of both glucosides is at its greatest at the end of the logarithmic phase of fruit development. The total content per fruit falls sharply when the corresponding neohesperidosides, naringin and neohesperidin, the most abundant flavanone glycosides in *C. aurantium*, reach their maximum levels. These data and the demonstration of the capacity of glucosyltransferase from cell-free extracts of *C. aurantium* tissues to glucosylate naringenin and hesperetin suggest that prunin and hesperetin 7-*O*-glucoside are direct precursors of naringin and neohesperidin, respectively, in *C. aurantium*.

INTRODUCTION

Citrus species are noted for their accumulation of flavanone glycosides in fruit and young vegetative tissues (Castillo et al., 1992; Horowitz and Gentili, 1963; Jourdan et al., 1985). Naringenin and hesperetin are most commonly found in *Citrus* species as the glycosides: naringin, neohesperidin (neohesperidosides), narirutin, and hesperidin (rutinosides) (Kamiya et al., 1979; Nishiura et al., 1971; Rouseff et al., 1987).

A study of the glucosylation of exogenous aglycons by undifferentiated *Citrus* cell cultures showed that when naringenin and hesperetin are used as acceptors, the products of the reactions are their 7-*O*-monoglucosides, prunin and hesperetin 7-*O*-glucoside, respectively (Lewinsohn et al., 1986, 1989a,b; McIntosh et al., 1990; McIntosh and Mansell, 1990). These data have led to the suggestion that these compounds might be precursors in the biosynthetic pathway leading to the formation of flavanone rhamnoglucosides in *Citrus* (Lewinsohn et al., 1989b) and that there is hydroxylation and methylation of naringenin to form hesperetin before glucosylation takes place; they also suggest that hesperetin is the precursor of neohesperidin (neohesperidoside) and hesperidin (rutinoside) (Lewinsohn et al., 1989b). However, hesperetin 7-*O*-glucoside has not been reported in natural *Citrus* species, and there are only two references to prunin in the immature fruits of *Citrus paradisi* (Berhow and Vandercook, 1989) and in the peels of mature fruits of zabon (*Citrus grandis* Osbeck) (Matsubara et al., 1986).

The absence of the free aglycons naringenin and hesperetin, and of their respective 7-*O*-glucosides, has been used to support the theory of a different flavonoid biosynthetic pathway in *Citrus* (Raymond and Maier, 1977). This theory suggests that the hydroxylation and methylation of naringenin occurs after glycosylation, and so neohesperidine and hesperidin would be synthesized from naringin and narirutin, respectively, by hydroxylation and subsequent methylation.

In this paper we demonstrate the natural occurrence of hesperetin 7-*O*-glucoside and prunin in *Citrus aurantium* and confirm the presence of prunin in *C. paradisi*. Hesperetin 7-*O*-glucoside was not detected in *Citrus sinensis*. We also report the quantitative distribution of prunin and hesperetin 7-*O*-glucoside during the development of *C. aurantium* fruits, the analysis of their respective levels compared with those of naringin and neohesperidin, their respective neohesperidosides, and the capacity of glucosyltransferase, isolated from *C. aurantium* tissues, to glucosylate naringenin and hesperetin. By this means we hope to clarify the biosynthetic pathway of glycosylflavanones in *Citrus* species and confirm the intermediary role of prunin and hesperetin 7-*O*-glucoside as suggested by other authors (Lewinsohn et al., 1989b; McIntosh et al., 1990; McIntosh and Mansell, 1990).

MATERIALS AND METHODS

Plant Material. Immature fruits of *C. aurantium* (4–61-mm diameter), *C. paradisi* (4–20-mm diameter), and *C. sinensis* (4–20-mm diameter) were obtained from 5-year-old trees grown in greenhouses of the University of Murcia.

Extraction of Flavonoids. The immature fruits were collected, immediately dried at 50 °C (Hosoda and Noguchi, 1988), and ground, and the flavonoids were extracted with DMSO in the ratio 10 mg/mL for analytical chromatography. For the isolation of prunin and hesperetin 7-*O*-glucoside, 50 g of dried fruits (5–20-mm diameter) was extracted with DMSO in the ratio 300 mg/mL. The solutions were filtered through a 0.45-μm nylon membrane.

Chromatographic Analysis. For the location of prunin and hesperetin 7-*O*-glucoside in the fruit extracts (*C. aurantium*, *C. paradisi*, and *C. sinensis*), we used a μBondapak C_{18} (250 × 4 mm i.d.) analytical column with an average particle size of 5 μm, and the solvents (1) isocratic water–methanol–acetonitrile–acetic acid (15:2:2:1) and (2) isocratic methanol–0.01 M phosphoric acid (1:3). The flow rate was 1 mL/min at room temperature. HPLC

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analysis was performed using a Beckman liquid chromatograph with a Model 110B solvent-delievery module and a System Gold Model 168 diode array detector. The absorbance change was monitored at 280 nm.

Isolation of Flavonoids. For the isolation of suspected prunin and hesperetin 7-*O*-glucoside, the following semipreparative column was used: Nucleosil C_{18} 5 μm (250 × 10 mm i.d.), eluted with methanol–0.01 M phosphoric acid (1:3) (solvent 2) at a flow rate of 5 mL/min. Both flavonoids were isolated by repeated chromatography in this semipreparative column and the fractions collected with a Pharmacia FRAC 100.

Identification of Flavonoids. The ^{1}H NMR (200 MHz) and ^{13}C NMR (50 MHz) spectra of two isolated flavonoids were obtained in hexadeuteriodimethyl sulfoxide (DMSO-d_6). Prunin (150 mg), from repeated semipreparative chromatography of plant material extracts (*C. aurantium* and *C. paradisi*), was hydrolyzed in 10 mL of 2 N sulfuric acid, under reflux for 1 h. A white precipitate was obtained. The cooled suspension was neutralized with NaOH and the precipitate filtered and washed with distilled water; 85 mg of a white precipitate was obtained. Hesperetin 7-*O*-glucoside (200 mg), from repeated semipreparative chromatography of plant material extracts (*C. aurantium*), was hydrolyzed in 50 mL of 2 N sulfuric acid, under reflux for 1 h. A white-beige precipitate was obtained. The cooled suspension was neutralized with NaOH and the precipitate filtered and washed with distilled water; 135 mg of a white-beige precipitate was obtained. The precipitates of both hydrolysis procedures were identified by their melting points, mass spectra (EIMS), and ^{1}H NMR (200 MHz) and ^{13}C NMR (50 MHz) spectra in hexadeuteriodimethyl sulfoxide (DMSO-d_6).

For the identification and isolation of the sugar in the neutralized hydrolysis solutions, an IR detector and the following semipreparative column was used: Nucleosil-NH_2 7 μm (250 × 10 mm i.d.), eluted with acetonitrile–water (85:15) at a flow rate of 4 mL/min at 30 °C (Jones et al., 1977). The sugar was isolated by repeated chromatography in this semipreparative column and the fraction collected with a Pharmacia FRAC 100. It was identified as glucose by comparing its mass spectrum (EIMS) with that of authentic sugar.

Preparation of Cell-Free Extracts of *C. aurantium* and UDPG: 7-*O*-Glucosyltransferase (GT) Assay. Cell-free extracts were obtained from *C. aurantium* fruits harvested at 43 days, as described previously for *Zea mays* (Larson, 1989), using the eluate of a Sephadex G-50 column as enzyme source. The protein content was determined according to the Bradford (1976) method.

The assay contained HEPES buffer (50 mM, pH 7.5) with 2 mM DTE, 200 μM UDP-glucose, 100 μM naringenin/hesperetin, and 2.8 mg of enzyme in 3 mL of total volume. Controls without substrate or enzyme were run with the samples and all incubated at 40 °C for 60 min. The assay was terminated by the addition of 200 μL of 2 N HCl. Product formation was determined by HPLC analysis of the reaction medium on μBondapak C_{18} (250 × 4 mm i.d.) with an average particle size of 5 μm, using methanol–water (40:60 v/v) as the eluent at a flow rate of 1 mL/min at room temperature. The column eluant was monitored at 280 nm.

RESULTS

HPLC analysis of the extracts of immature fruits of *C. aurantium* shows two compounds with retention times identical to those of prunin and hesperetin 7-*O*-glucoside in two chromatographic systems (Table I), whereas the extracts of immature fruits of *C. paradisi* show one compound with a retention time identical to that of prunin in two chromatographic systems (Table I). Analysis, under similar conditions, of extracts of immature fruits of *C. sinensis* did not show any flavonoids with retention times identical to those of prunin or hesperetin 7-*O*-glucoside.

The suspected glucosides were isolated to confirm their identities. Their absorption spectra showed two maxima (in elution solvent 2) at 283 and 329 nm, for a compound with a retention time identical to that of prunin, and at 284 and 327 nm for a compound with a retention time identical to that of hesperetin 7-*O*-glucoside. These data

Table I. Retention Times (Minutes) of Glycosylflavanones Present in *C. aurantium* and Simultaneously in *C. sinensis* or *C. paradisi*

flavonoid	eleuent 1[a]	flavonoid	eluent 2[b]
neoeriocitrin	12.02	neoeriocitrin	38.46
narirutin	17.35	prunin	53.28
prunin	20.01	narirutin	57.21
naringin	20.33	naringin	70.65
hesperidin	24.50	hesperetin 7-*O*-glucoside	84.47
hesperetin 7-*O*-glucoside	29.05	hesperidin	92.98
neohesperidin	29.34	neohesperidin	114.23

[a] Water–methanol–acetonitrile–acetic acid 15:2:2:1. [b] Isocratic methanol–0.01 M phosphoric acid 1:3.

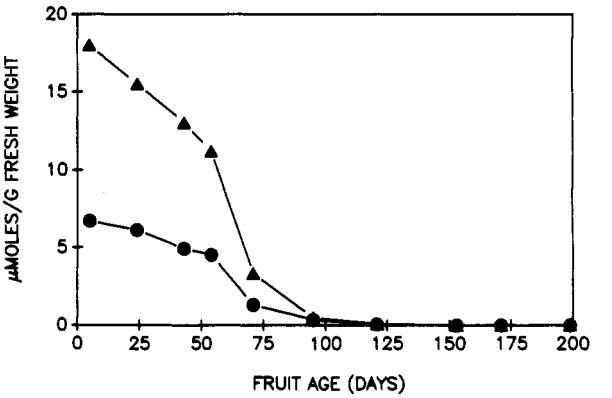

Figure 1. Accumulation of prunin (●) and hesperetin 7-*O*-glucoside (▲) concentration according to age in *C. aurantium* fruit. Vertical bars indicate ±SE when larger than symbols.

are consistent with the compounds' having flavanone skeletons identical to those of flavanone glucosides (Mabry et al., 1970a). These compounds were isolated with a semipreparative column (see Materials and Methods) and two white-yellow products obtained. Both ^{1}H NMR and ^{13}C NMR spectra for these compounds corresponded to a flavanone structure (Mabry et al., 1970b), showing glucosylation at position 7.

Hydrolysis of these compounds in 2 N sulfuric acid gave a white precipitate (for prunin) and a white-beige precipitate (for hesperetin 7-*O*-glucoside). These precipitates had UV spectra, melting points, and mass, ^{1}H NMR, and ^{13}C NMR spectra identical to those of the flavanones naringenin and hesperetin, respectively. The sugar resulting from hydrolysis was isolated by semipreparative chromatography (see Materials and Methods) and identified as glucose by comparing its mass spectrum with that of authentic sugar.

These data led us to believe that the compounds in question referred to prunin and hesperetin 7-*O*-glucoside (Lewinsohn et al., 1986; Mabry et al., 1970; Matsubara et al., 1986).

Changes in the Levels of Prunin and Hesperetin 7-*O*-Glucoside during Development of *C. aurantium* Fruits and Their Relation with Those of Naringin and Neohesperidin, Respectively. Growth of bitter orange fruit, as measured by fruit diameter, is sigmoidal, reaching its maximum size (61-mm diameter) at approximately 200 days. From this time, the processes of maturation begin, with no appreciable change in diameter, as described previously (Castillo et al., 1992).

Prunin and hesperetin 7-*O*-glucoside concentrations (Figure 1) are higher in the first stages of development coinciding with the logarithmic phase of fruit growth. These concentrations decrease more rapidly for hesperetin 7-*O*-glucoside than for prunin as the fruit grow. After 170

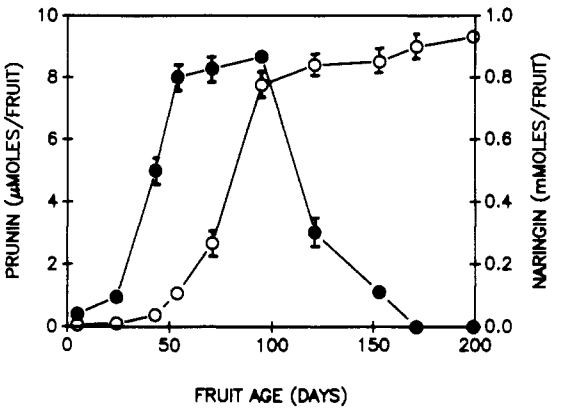

Figure 2. Changes in mean total content per fruit of prunin (●) and naringin (○) according to age in *C. aurantium*. Vertical bars indicate ±SE when larger than symbols.

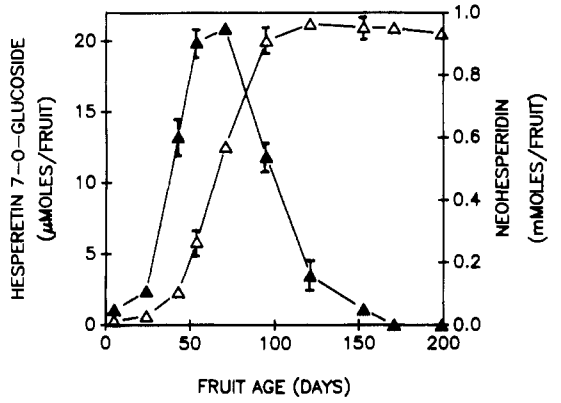

Figure 3. Changes in mean total content per fruit of hesperetin 7-*O*-glucoside (▲) and neohesperidin (△) according to age in *C. aurantium*. Vertical bars indicate ±SE when larger than symbols.

days, neither glucoside was detectable by the techniques used, such was the huge decrease they had undergone.

Three zones could be differentiated in the accumulation curve of both flavonoids (Figures 2 and 3): an initial stage up to 60 days, coinciding with the logarithmic phase of fruit growth, and in which a huge accumulation or relative synthesis was produced, reaching a maximum level at the end of this period; a second stage, coinciding with the initial phase of linear growth (60–100 days) in which the level reached remained practically constant; and a third stage in which the accumulation of these glucosides ceased before decreasing sharply during the final stages of the linear phase of growth and the beginning of maturation.

During the development of *C. aurantium* fruits, the total content per fruit of naringin and neohesperidin is always more than that of the respective glucosides. however, it must be stated that at the beginning of the fruit growth (3–5-mm diameter) the neohesperidoside content is very similar to that of the pollinated flower ovary after the fall of petals (Castillo et al., 1992), whereas the glucoside content can only be detected when, after this pollination, the fruit begins to grow.

The analysis of DMSO extracts of *C. aurantium* fruits during their development shows small quantities (0.1 μmol/g of fresh weight) of hesperetin, the immediate precursor of hesperetin 7-*O*-glucoside biosynthesis, and no naringenin. Using different scales for the coordinates, Figures 2 and 3 show how the first step of the distribution curve of both glucosides coincided with the latency and accumulation stages of their respective neohesperidosides. The second step of the distribution curves coincides with an increase in neohesperidoside synthesis or accumulation rate. When both neohesperidosides have reached their maximum levels, the respective glucosides present a sharp decrease until they cannot be detected by the methods used.

An analysis of the relationship between the hesperetin 7-*O*-glucoside and prunin levels during fruit development in *C. aurantium* shows, significantly, values similar to those of the ratio between neohesperidin and naringin (Figures 2 and 3).

Analysis of Naringenin and Hesperetin Glucosylation by Cell-Free Extracts of *C. aurantium* Tissues. To establish the origin of prunin and hesperetin 7-*O*-glucoside, enzyme was extracted as indicated under Materials and Methods. Neither of the flavonoids was detected in the enzymatic extracts obtained. Our experiments confirm that both naringenin and hesperetin are glucosylated by cell-free extracts of *C. aurantium* to prunin and hesperetin 7-*O*-glucoside, respectively. These results coincide with those for other *Citrus* species (Lewinsohn et al., 1989b; McIntosh et al., 1990; McIntosh and Mansell, 1990) and for cell suspension cultures of *C. aurantium* (Lewinsohn et al., 1989a). However, glucosyltransferase activity obtained from cell-free extracts of *C. aurantium* is higher than that obtained from the enzymes isolated from *C. paradisi* (McIntosh et al., 1990; McIntosh and Mansell, 1990) and *Citrus mitis* (Lewinsohn et al., 1989b). The specific activities for naringenin and hesperetin are also seen to be similar.

DISCUSSION

In this work, we have used a profile protocol to examine the distribution of the glucosylflavanones prunin and hesperetin 7-*O*-glucoside in *C. aurantium* fruits during their development. This species accumulates the highest quantities of both glucosides. Similarly, the occurrence of these products in immature fruits of *C. paradisi* and *C. sinensis* has been examined.

The main aims of this paper are to describe the natural occurrence and levels of prunin and hesperetin 7-*O*-glucoside during the development of *C. aurantium* fruits and, more importantly, to attempt to establish the biosynthetic pathway of these glucosides in the plant tissues studied by analyzing the capacity of cell-free extracts of *C. aurantium* to glycosylate naringenin and hesperetin. In this way the role of these compounds in the biosynthesis pathways of the two most abundant flavanone neohesperidosides in *C. aurantium*, naringin and neohesperidin, may be established.

The relative levels of these glucosides, prunin and hesperetin 7-*O*-glucoside, are markedly affected by the age of the developing fruit. Our results demonstrate that the concentration of both flavonoids is greater in young tissues and that there is a sharp decrease during linear growth. This behavior is somewhat similar to that of their neohesperidosides in the same fruit (Castillo et al., 1992) and to that described for other flavanones in *C. paradisi* (Jourdan et al., 1985) and *Citrus limonia* (Vandercook and Tisserat, 1989).

However, the decrease in concentration observed, as stated under Results, is due to dilution by water and other compounds which enter the enlarging cells (Castillo et al., 1992; Jourdan et al., 1985), mainly in the logarithmic phase of fruit growth, since the total content per fruit of prunin and hesperetin 7-*O*-glucoside increases during this phase. Both glucosides have practically disappeared by the time the fruit reaches maximum development.

We have demonstrated that cell-free extracts of *C. aurantium* immature fruits are capable of glucosylating

Table II. Glucosyltransferase Activity of Cell-Free Extracts of *C. aurantium* for Naringenin and Hesperetin

substrate	TA,[a] pmol/s	SA,[b] pmol s^{-1} (mg of protein)$^{-1}$	product
naringenin	16.666	1.98	prunin
hesperetin	15.739	1.87	hesperetin 7-*O*-glucoside
control[c]	0.000	0.00	NA[d]

[a] Total activity. [b] Specific activity. [c] Control without substrate or enzyme. [d] Not applicable.

naringenin and hesperetin to prunin and hesperetin 7-*O*-glucoside, respectively, with similar specific activities (Table II), thus confirming that the presence of these glucosides is not due to a possible degradative hydrolytic process (until now undescribed in *Citrus*) of their corresponding neohesperidosides, naringin and neohesperidin.

C. aurantium fruits accumulate, during their development, large quantities of these neohesperidosides (Castillo et al., 1992), and these quantities are always much higher than those of any other flavanone or flavone whose presence has also been described in this material. This implies high activity on the part of all those enzymes involved in the biosynthesis pathways of both neohesperidosides: chalcone-flavanone synthase, hydrolases, methyltransferases, and enzymes of glycosylation. The natural occurrence of glucosides referred to in this paper affords important information with regard to the biosynthetic pathway of the neohesperidosides accumulated in *C. aurantium*.

The presence of prunin in *C. aurantium* and *C. paradisi* may not be conclusive in determining which of the several routes described for flavanone 7-*O*-rhamnoglucoside biosynthesis in *Citrus* species (Lewinsohn et al., 1989b; Raymond and Maier, 1977) is the definitive one, since the above glucosides might exist as intermediates, with no defined stability, in naringin biosynthesis. According to some authors (Raymond and Maier, 1977) the selecting activity of chalcone cyclase from immature grapefruit and other data suggest that the intermediates which form the B-ring of chalcones are hydrolyated prior to chalcone formation, that chalcones are glycosylated during their formation, a period in which the flavanone glucoside, prunin, might exist as a result of chalcone–flavanone equilibrium, and that B-ring methylation occurs after flavanone rhamnoglucoside formation.

This biosynthetic pathway precludes the existence of hesperetin 7-*O*-glucoside. However, the natural occurrence of this glucoside and the confirmation of hesperetin glucosylation by glucosyltransferase isolated from *C. aurantium* tissues do suggest that B-ring hydroxylation and methylation reactions occur in the C_{15} flavanone skeleton stage, that is, the biosynthetic pathway of neohesperidin takes place through naringenin and the hydroxylation and methylation of this flavanone aglycon to form hesperetin occurs before glucosylation. This is in agreement with other studies in *C. mitis* and *Citrus maxima* (Lewinsohn et al., 1989b) (although in these species the natural occurrence of prunin and hesperetin 7-*O*-glucoside has not been described previously) and *C. paradisi* (Berhow and Vandercook, 1989; McIntosh et al., 1990; McIntosh and Mansell, 1990).

Taking into account that the biosynthetic pathway of the flavanone neohesperidosides is as described in Figure 4, the absence of the aglycon naringenin, the small

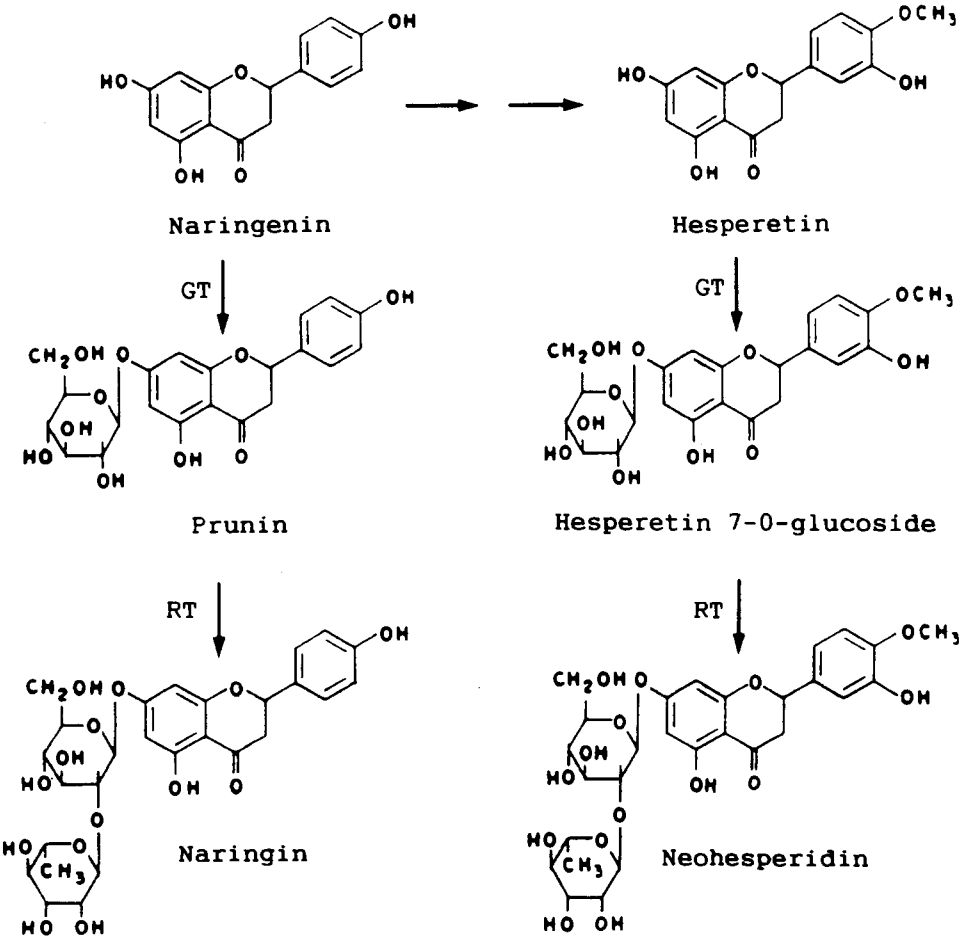

Figure 4. Proposed biosynthetic pathways from naringenin to naringin and neohesperidin in *C. aurantium*. Enzymes involved were glucosyltransferase (GT) and rhamnosyltransferase (RT).

quantities of hesperetin, and the scant accumulation of the glucosides prunin and hesperetin 7-*O*-glucoside suggest that the glucosylation and rhamnosylation enzymes are more active than the other enzymes involved in this biosynthetic pathway, particularly chalcone synthase. The fact that there is a slight accumulation of the glucosides prunin and hesperetin 7-*O*-glucoside during the first stages of fruit development (logarithmic phase) might be related to a possible minimum delay in the induction period or in the timing of maximum activity between glucosyltransferase and rhamnosyltransferase enzymes.

Figures 2 and 3 show that the ratio of neohesperidin/naringin levels decreases during fruit development to 1.00, since the total quantities per fruit of both neohesperidosides, when the maturation phase begins, are similar. Since naringenin is the first flavanone involved in the biosynthetic pathway of both neohesperidosides, the decrease in the above ratio might be due to a progressive diminution of the relative activity of the enzymes involved in the biosynthesis of hesperetin, particularly of the enzyme 4′-*O*-methyltransferase, recently isolated and characterized by the authors in *C. aurantium* tissues (unpublished results). The fact that the evolution of the ratios of the neohesperidoside levels is very similar to those of respective glucoside level ratios suggests that a constant and direct proportionality exists between the glucosylation rates of flavanone aglycons and the rhamnosylation rates of their flavanone glucosides. This seems to suggest the existence of a regulatory mechanism for both biosynthetic steps.

The absence of prunin and hesperetin 7-*O*-glucoside in extracts of immature fruits of *C. sinensis* might be related to the different activities of the enzymes involved in the subsequent rhamnosylation of these compounds, since in *C. paradisi* and *C. aurantium* the glucose–rhamnose bond is of the neohesperidoside type [1–2] and in *C. sinensis* of the rutinoside type [1–6].

The final conclusion is that the fruits of *C. aurantium* trees are capable of synthesizing or accumulating prunin and hesperetin 7-*O*-glucoside during the stage of cell differentiation, which disappears at the end of the linear growth stage and during the subsequent maturation period. It can also be concluded that prunin and hesperetin 7-*O*-glucoside are probably the immediate precursors of naringin and neohesperidin, respectively, in *C. aurantium*. In addition, we report for the first time the presence of hesperetin in *Citrus*.

Studies on the complete characterization of glucosyltransferase and rhamnosyltransferase in *C. aurantium* tissues are in progress.

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LITERATURE CITED

Berhow, M. A.; Vandercook, C. E. Biosynthesis of naringin and prunin in detached grapefruit. *Phytochemistry* **1989**, *28*, 1627–1630.

Bradford, M. M. A rapid and intensive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248–254.

Castillo, J.; Benavente-Garcia, O.; Del Rio, J. A. Naringin and neohesperidin levels during development of leaves, flower buds and fruits of *Citrus aurantium*. *Plant Physiol.* **1992**, *99*, 67–73.

Horowitz, R. M.; Gentili, B. Flavonoids of Citrus. VI. The structure of Neohesperidose. *Tetrahedron* **1963**, *19*, 773–782.

Hosoda, K.; Noguchi, M. Studies on the preparation and evaluation of kijitsu, the immature *Citrus* fruits I. Evaluation of a new drying method. *J. Pharm. Soc. Jpn.* **1988**, *108*, 1008–1011.

Jones, A. D.; Burns, I. W.; Sellings, S. G.; Cox, J. A. Preparation, optimisation and slurry packing of an amino bonded phase for the analysis of sugars in food by high-performance liquid chromatography. *J. Chromatogr.* **1977**, *144*, 169–180.

Jourdan, P. S.; McIntosh, C. A.; Mansell, I. W.; Sellings, S. G.; Preparation, optimisation and slurry packing of an amino bonded phase for the analysis of sugars in food by high-performance liquid chromatography. *J. Chromatogr.* **1977**, *144*, 169–180.

Jourdan, P. S.; McIntosh, C. A.; Mansell, R. L. Naringin levels in Citrus tissues. II. Quantitative distribution of naringin in *Citrus paradisi* Macfad. *Plant Physiol.* **1985**, *77*, 903–908.

Kamiya, S.; Esaki, S.; Konishi, F. Flavonoids in *Citrus* hybrids. *Agric. Biol. Chem.* **1979**, *43*, 1529–1536.

Larson, R. L. Flavonoids 3′-*O*-methylation by a *Zea mays* L. preparation. *Biochem. Physiol. Pflanzen.* **1989**, *184*, 453–460.

Lewinsohn, E.; Berman, E.; Mazur, Y.; Gressel, J. Glucosylation of exogenous flavanones by grapefruit (*Citrus paradisi*) cell cultures. *Phytochemistry* **1986**, *25*, 2531–2535.

Lewinsohn, E.; Berman, E.; Mazur, Y.; Gressel, J. (7)-Glucosylation and (1–6) rhamnosylation of exogenous flavanones by ondefferentiated *citrus* cell cultures. *Plant Sci.* **1989a**, *61*, 23–28.

Lewinsohn, E.; Britsch, L.; Mazur, Y.; Gressel, J. Flavanone Glucoside biosynthesis in *Citrus*. *Plant Physiol.* **1989b**, *91*, 1323–1328.

Mabry, T. J.; Markham, K. R.; Thomas, M. B. The UV spectra of isoflavones, flavanones, and dihidroflavonols. In *The Systematic identification of flavonoids*; Springer-Verlag: New York, 1970a.

Mabry, T. J.; Markham, K. R.; Thomas, M. B. The NMR spectra of flavonoids. In *The Systematic identification of flavonoids*; Springer-Verlag: New York, 1970b.

Matsubara, Y.; Yonemoto, H.; Kunamoto, H.; Iizuka, Y.; Okamoto, K.; Yokoi, K. Flavonoid Glycosides in *Citrus* Fruit Peelings. *Nippon Nogeikagaku Kaishi* **1986**, *35*, 435–437.

McIntosh, C. A.; Mansell, R. L. Biosynthesis of naringin in *Citrus paradisi*: UDP-glucosyltransferase activity in grapefruit seedlings. *Phytochemistry* **1990**, *29*, 1533–1538.

McIntosh, C. A.; Latchinian, L.; Mansell, R. L. Flavanone specific 7-O-Glucosyltransferase Activity in *Citrus paradisi* seedlings: Purification and characterization. *Arch. Biochem. Biophys.* **1990**, *282*, 50–57.

Nishiura, M.; Kamiya, S.; Esaki, S. Flavonoids in *Citrus* and related Genera. Part III. Flavonoid Pattern and Citrus Taxonomy. *Agric. Biol. Chem.* **1971**, *35*, 1691–1706.

Raymond, W. R.; Maier, V. P. Chalcone cyclase and flavonoid biosynthesis in grapefruit. *Phytochemistry* **1977**, *16*, 1535–1539.

Rouseff, R. L.; Martin, S. F.; Youtsey, C. O. Quantitative survey of narirutin, naringin, hesperidin and neohesperidin in *Citrus*. *J. Agric. Food Chem.* **1987**, *35*, 1027–1030.

Vandercook, C. E.; Tisserat, B. Flavonoids changes in developing lemons grown in vivo and in vitro. *Phytochemistry* **1989**, *28*, 799–803.

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