Glycosidically Bound Eugenol and Methyl Salicylate in the Fruit of Edible *Passiflora* Species

David Chassagne,*,† Jean Crouzet,† Claude L. Bayonove,‡ and Raymond L. Baumes‡

Laboratoire de Génie Biologique et Sciences des Aliments, Unité de Microbiologie et Biochimie Industrielles, Associée à l'INRA, Université de Montpellier II, 34095 Montpellier Cedex 05, France, and Laboratoire des Arômes et des Substances Naturelles, Institut des Produits de la Vigne, INRA, 2 Place Viala, 34060 Montpellier Cedex 01, France

The β-D-glucopyranoside and 6-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside of methyl salicylate and the β-D-glucopyranoside of eugenol have been characterized in purple passion fruit (*Passiflora edulis* SIMS) by GC and GC/MS of their trifluoroacetylated derivatives. The structural elucidations were performed by comparison of chromatography and mass spectrometry data with those of synthesized glycosides. Identifications and (semi)quantifications of some of them have been extended to yellow passion fruit (*Passiflora* f *flavicarpa*), granadilla (*Passiflora ligularis* Juss.), and banana passion fruit (*Passiflora mollissima*).

Keywords: *Passion fruit; phenolic glycosides; gas chromatography; mass spectrometry*

INTRODUCTION

During the past two decades the determination of glycosidically bound aroma compounds in fruits and different plant species has attracted considerable research interest (Cordonnier and Bayonove, 1974; Williams et al., 1981; Stahl-Biskup, 1987; Stahl-Biskup et al., 1993). Aroma compounds could be released from these nonvolatile precursors during maturation, pretreatment, or processing by enzymic or acid hydrolysis. For yellow passion fruit, monoterpenic, aliphatic, and aromatic alcohol content was considerably increased during acid-catalyzed treatment of juice (Engel and Tressl, 1983). In addition, C_{13} norisoprenoids have been identified after enzymic hydrolysis of purple passion fruit glucosidic extracts (Winterhalter, 1990). Studies provided by our group have allowed the identification in purple passion fruit of multiple monoglucosides and diglycosides as aroma precursors (Chassagne et al., 1995a; Chassagne, 1996), particularly, 6-*O*-α-L-arabinopyranosyl-β-D-glucopyranosides (β-vicianosides) of (*S*)-linalool, benzyl alcohol, and 3-methylbut-2-en-1-ol (Chassagne et al., 1996a). In addition, prunasin and amygdalin have been characterized and quantified in the juice and peel of four edible *Passiflora* species (Chassagne et al., 1996b).

In the course of our own studies of passion fruit glycosides, we have noticed the presence of several phenolic volatiles released by enzymic hydrolysis of glycosidic extracts, in particular eugenol and methyl salicylate (Chassagne et al., 1995a). Among the two last compounds, only eugenol has been reported as a volatile compound in yellow passion fruits (Winter and Kloti, 1972). Considering the contribution of phenolic compounds to the overall flavor of fruits, we decided to pursue the investigation of their glycosidic forms. This paper reports the identification of three phenolic glycosides and their (semi)quantification in four *Passiflora* species.

* Author to whom correspondence should be addressed [fax 00(33)467144292; e-mail chassagne@arpb.univ-montp2.fr].

† Université de Montpellier II.

‡ INRA.

MATERIALS AND METHODS

Fruits. *Passiflora edulis* (purple passion fruit) from Zimbabwe and *Passiflora ligularis* from Colombia were purchased at Rungis market (Paris, France). *P. edulis* f *flavicarpa* (yellow passion fruit) were grown in the Centre de Recherches IRA Nyombé (Cameroon), and the *Passiflora mollissima* harvested on Réunion Island were supplied by CIRAD.

Sample Preparation. With the exception of *P. mollissima*, fruits were cut, and the pulp was filtered through gauze to remove the seeds. Then the pulp was centrifuged (30 min; 10000*g*) at 4 °C to obtain a clear juice. For *P. mollissima*, fruits were sliced and crushed in a Waring Blendor for 2 min in the presence of deionized water (1:1 w/v). The homogenate was centrifuged under the conditions indicated above.

Passion fruit peel was extracted by crushing with methanol (1:2 w/v) in a Waring Blendor for 2 min. After 20 min of contact, the mixture was filtered under vacuum. The methanol was evaporated under vacuum at 45 °C, and the residue was suspended in deionized water.

Isolation of Natural Glycosidic Components. The sample, corresponding to 50 mL of clear juice, 50 g of peel, or 50 g of fruits for *P. mollissima*, was passed through a column (90 × 10 mm) of Amberlite XAD-2 resin (20−60 mesh, Rhöm and Haas, Chauny, France) following the method of Günata et al. (1985). After washing of the column with 50 mL of deionized water and 50 mL of pentane/dichloromethane (2:1 v/v), the glycosidic extract was obtained by eluting with 50 mL of methanol.

Enzymic Hydrolysis. The glycosidic extracts obtained from 45 mL of clear juice or 45 g of peel of *P. edulis* were concentrated in vacuum to dryness, redissolved in 0.3 mL of 0.2 M citrate−phosphate buffer (pH 5), and washed five times using pentane/dichloromethane (2:1). To the residue was added 0.3 mL of an enzymic preparation [12.5 mg of hemicellulase REG-2 (Gist Brocades, Seclin, France) and 20 mg of almond glucosidase (Boehringer Mannheim) in 1 mL of 0.2 M citrate−phosphate buffer (pH 5)]. Then the mixture was incubated at 40 °C for 16 h. After the mixture had cooled to room temperature, 32 μg of 4-nonanol was added as internal standard and the mixture was extracted five times with 300 μL of pentane/dichloromethane (2:1). This aglycon extract was concentrated to a final volume of 300 μL by microdistillation at 37 °C.

Trifluoroacetylation of Glycosides. The bound fraction obtained from 0.5 mL of juice, 0.5 g of peel, 0.5 g of berry, or a mixture of synthetic glycosides was concentrated to dryness, in a small screw-capped vial at 60 °C under nitrogen, and derivatized with the TFA reagent [*N*-methyl-bis(trifluo-

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roacetamide)] according to the method of Voirin et al. (1992). Phenyl β-D-glucopyranoside (10 μg) was used as an internal standard.

GC Analysis. Varian Model 3300 gas chromatographs equipped with split injector (1/10) and a flame ionization detector were used.

For aglycons, two types of fused silica capillary columns were employed: J&W Scientific (Folsom, CA) DB-Wax (a) and DB-5MS (b) (30 m × 0.25 mm i.d., film thickness = 0.25 μm). The temperature programs were (a) 3 min isothermal at 60 °C and then increased at 2 °C/min to 220 °C and (b) 40 °C increased to 200 °C at 2 °C/min. The flow rates (a and b) were 1.8 mL/min of H_2 for the carrier gas, 30 mL/min N_2 for the makeup gas, and 30 mL/min of H_2 and 300 mL/min of air for the detector gases. The injector temperature (a and b) was maintained at 250 °C and the detector temperature at 250 (a) and 300 °C (b).

For trifluoroacetylated glycosides, a DB-5MS fused silica capillary column was used (30 m × 0.25 mm i.d., film thickness = 0.25 μm, J&W Scientific). The column temperature was programmed at 3 °C/min from 125 to 220 °C, then increased at 2 °C/min to 280 °C, and held at this temperature for 15 min. The flow rates for the carrier gas (H_2), the makeup gas, and detector gases were the same as those mentioned above. The injector temperature was maintained at 280 °C and the detector temperature at 300 °C.

GC/MS Analysis of Trifluoroacetylated Glycosides. Mass spectra were recorded by coupling a Hewlett-Packard (HP) 5890 gas chromatograph equipped with a DB-5MS fused silica capillary column (30 m × 0.25 mm i.d., film thickness = 0.25 μm) and an injector on column to a HP 5889A mass spectrometer. The transfer line was heated at 290 °C, and the injector temperature was programmed at 60 °C/min from 110 to 260 °C and then held at this temperature for 55 min. The column temperature was programmed at 3 °C/min from 125 to 290 °C with helium as carrier gas at 1.1 mL/min. EI-MS was performed at 70 eV and NCI-MS at 200 eV with methane as reagent gas at 80 Pa, according to the procedure described by Chassagne et al. (1995b).

NMR Analysis. NMR spectra were recorded with a Bruker 250 MHz spectrometer (250 MHz for ^{1}H NMR and 62.89 MHz for ^{13}C NMR) in chloroform-d_1 for acetylated glycosides and water-d_2 for glycosides (internal standard, tetramethylsilane).

Synthesis of 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosides. Glucosides (**1b**, **2b**) were synthesized under the experimental conditions described by Baumes et al. (1989).

2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranoside bromide (1.95 mmol) and freshly prepared Ag_2CO_3 (6.5 mmol) were added under stirring to volatile compounds (3 mmol) and 1 g of drierite in 20 mL of anhydrous pyridine. The mixture was stirred in the dark for 48 h at room temperature and then filtered under vacuum. The filtrate was concentrated under vacuum at 50 °C and then dissolved in 50 mL of toluene and concentrated again to eliminate pyridine traces. The crude product was dissolved in 100 mL of diethyl ether, and washed with ice-cold water, and dried with Na_2SO_4. After filtration and concentration, the final crude product was purified by column chromatography on silica gel (230−400 mesh, Merck) using diethyl ether/petroleum ether (8:2) as solvent.

2,3,4,6-Tetra-O-acetyl-β-D-glucopyranoside of methyl salicylate (***1b***): ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.95, 1.97, 1.99, 2.02 (each CH_3CO), 3.87 (s, 3H, H-8), 3.90 (ddd, *J* = 9.7, 5.1, 2.5 Hz, H-5′), 4.19 (dd, *J* = 12.3, 2.5 Hz, H_b-6′), 4.31 (dd, *J* = 12.3, 5.1 Hz, H_a-6′), 5.12 (d, *J* = 7.6 Hz, H-1′), 5.20 (m, H-4′), 5.24−5.34 (m, H-2′), 5.37 (dd, *J* = 9.0, 8.1 Hz, H-3′), 7.14 (dd, *J* = 8.0, 7.2 Hz, H-4), 7.15 (d, *J* = 8.0 Hz, H-6), 7.47 (ddd, *J* = 8.0, 7.2, 1.7 Hz, H-5), 7.78 (dd, *J* = 8.0, 1.7 Hz, H-3).

Eugenyl 2,3,4,6-Tetra-O-acetyl-β-D-glucopyranoside (***2b***): ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.96−2.01 (4 s, CH_3CO), 3.27 (d, 2H, *J* = 6.6 Hz, H-7), 3.67 (ddd, *J* = 10.0, 5.0, 2.5 Hz, H-5′), 3.74 (s, 3H, H-10), 4.09 (dd, *J* = 12.2, 5.0 Hz, Ha-6′), 4.22 (dd, *J* = 12.2, 2.5 Hz, Hb-6′), 4.85 (d, J = 7.7 Hz, H-1′), 4.96−513 (m, 3H, H-2′, H-3′, H-4′), 5.21 (m, 2H, H-9), 5.87 (m, *J* = 16.5, 11.0, 6.6 Hz, H-8), 6.63 (dd, *J* = 8.0, 2.6 Hz, H-5), 6.65 (m, H-3), 6.97 (d, *J* = 8.0 Hz, H-6).

Deacetylation of Peracetylated Glucosides. To peracetylated glucosides dissolved in 2 mL of methanol was added 0.1 mL of 0.3 M sodium methoxide in methanol at room temperature. The mixture was stirred for 90 min at room temperature, then neutralized by adding Zerolit 225 (H^+ form) resin, and filtered. The crude product was purified by column chromatography on silica gel (Merck, 230−400 mesh) using ethyl acetate/ethanol (3:1) as solvent.

β-D-Glucopyranoside of methyl salicylate (***1c***): ^{1}H NMR of (250 MHz, D_2O) δ 3.64−3.84 (m, 4H, H-2′, H-3′, H-4′, H-5′), 3.90 (dd, *J* = 12.4, 5.2 Hz, H_a-6′), 4.05 (s, 3H, H-8), 4.07 (dd, *J* = 12.4, 2.3 Hz, H_b-6′), 5.30 (d, *J* = 7.7 Hz, H-1′), 7.36 (dd, *J* = 7.8, 7.8 Hz, H-4), 7.46 (d, *J* = 8.3, H-6), 7.76 (ddd, *J* = 8.3, 7.8, 1.7 Hz, H-5), 7.95 (dd, *J* = 7.8, 1.7 Hz, H-3).

Eugenyl β-D-glucopyranoside (***2c***): ^{1}H NMR (250 MHz, D_2O) δ 3.24 (d, *J* = 6.7 Hz, H-7), 3.48 (m, 4H, H-2′, H-3′, H-4′, H-5′), 3.64 (dd, *J* = 12.4, 5.0 Hz, H_a-6′), 3.76 (s, 3H, H-10), 3.80 (dd, *J* = 12.4, 1.4 Hz, H_b-6′), 4.97 (m, H-9), 4.98 (d, *J* = 7.7 Hz, H-1′), 5.92 (m, H-8), 6.75 (dd, *J* = 8.3, 1.6 Hz, H-5), 6.88 (d, *J* = 1.6 Hz, H-3), 7.02 (d, *J* = 8.3 Hz, H-6).

Synthesis of β-Rutinoside. 1,2,3,4-Tetra-*O*-acetyl-6-*O*-(2′,3′,4′-tri-*O*-acetyl-α-L-rhamnopyranosyl)-β-D-glucopyranoside (1 mmol) and 0.2 mL of acetic anhydride were stirred at −4 °C in 10 mL of chloroform, and then 1.8 mL of hydrobromic acid (33% in acetic acid) was added dropwise. After stirring under N_2 for 3 h, the mixture was poured into 15 mL of ice-cold water, then dried with Na_2SO_4, and concentrated under vacuum at 35 °C. The crude heptaacetyl α-bromorutinoside was used in the next step without further purification. Synthesis and deacetylation of **3b** were performed as described for **1b** and **2b**.

2,3,4-Tetra-O-acetyl-6-O-(2′,3′,4′-tri-O-acetyl-α-L-rhamnopyranosyl)-β-D-glucopyranoside of methyl salicylate (***3b***): ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.12 (d, *J* = 6.3 Hz, H-6″), 1.94−2.02 (6s, each CH_3CO), 3.52 (m, 1H, H-5′), 3.58 (dd, *J* = 11.5, 7.2 Hz, H_a-6′), 3.68 (dd, *J* = 11.5, 2.3 Hz, H_b-6′), 3.78 (s, 3H, H-8), 3.82 (m, 1H, H-5″), 4.68 (s, 1H, H-1″), 4.98 (m, 2H, *J* = 8.7, 7.7 Hz, H-2′, H-4′), 5.04 (d, *J* = 7.7 Hz, H-1′), 5.17−5.32 (m, 4H, H-3′, H-2″, H-3″, H-4″), 7.04 (dd, *J* = 7.8, 7.8 Hz, H-4), 7.06 (d, *J* = 7.8 Hz, H-6), 7.48 (ddd, *J* = 7.8, 7.8, 1.7 Hz, H-5), 7.67 (dd, *J* = 7.8, 1.7 Hz, H-3).

6-O-α-L-Rhamnopyranosyl-β-D-glucopyranoside of methyl salicylate (***3c***): ^{1}H NMR (250 MHz, D_2O) δ 1.15 (d, *J* = 6.2 Hz, H-6″), 3.37 (t, *J* = 9.6 Hz, H-4″), 3.50 (t, *J* = 9.1 Hz, H-5′), 3.60 (t, *J* = 8.9 Hz, H-3′), 3.64 (m, 2H, H-2′, H-4′), 3.70 (m, 2H, Ha-6′, H-5″), 3.75 (dd, *J* = 9.7, 3.4 Hz, H-3″), 3.87 (m, 1H, H-2″), 3.89 (s, 3H, H-8), 3.99 (d, *J* = 9.8 Hz, H_b-6′), 4.74 (d, *J* = 1.3 Hz, H-1″), 5.15 (d, *J* = 7.3 Hz, H-1′), 7.20 (dd, *J* = 7.8, 7.8 Hz, H-4), 7.28 (d, *J* = 8.4 Hz, H-6), 7.64 (ddd, *J* = 8.4, 7.8, 1.6 Hz, H-5), 7.78 (dd, *J* = 7.8, 1.6 Hz, H-3).

RESULTS AND DISCUSSION

Glycosidic extracts from juice or peel of fruits of *P. edulis* were purified by selective retention on Amberlite XAD-2 resin (Günata et al., 1985). After elution with methanol, the glycosidic fractions were hydrolyzed with hemicellulase REG-2 and almond glucosidase mixture as indicated by Chassagne et al. (1995a) to release the aglycons from passion fruit aroma precursors. GC/MS of the liberated volatile compounds revealed the occurrence of eugenol and methyl salicylate by comparison of linear retention index and MS data using authentic compounds. Eugenol was very abundant in juice (920 μg/g) and peel (1700 μg/kg), whereas methyl salicylate was abundant in only juice (760 μg/kg). However, there is no information on the sugar moiety of these glycosidically bound phenolic compounds. Thus, these phenolic glycosides have been characterized by means of GC and GC/MS analyses of their trifluoroacetylated derivatives (Voirin et al., 1992). The GC of TFA derivatives of passion fruit juice and peel glycosides showed many peaks, as illustrated in Figure 1 for the purple passion fruit peel extract; most of them were

Table 1. Mass Spectra of Trifluoroacetylated Derivatives of Phenolic Glycosides Detected in *P. edulis* Fruit Extracts (N) and Synthesized (S)

compd[a]		RI[b]	EIMS characteristic fragment ions of sugar moiety	EIMS characteristic fragment ions of aglycon moiety
1a	S	1970	319 (17), 177 (11), 193 (5), 205 (5)	152 (100), 120 (55), 69 (33), 109 (10), 92 (10)
	N	1963	319 (23), 177 (14), 193 (6), 205 (6)	152 (100), 69 (55), 120 (55), 92 (13), 109 (12)
2a	S	2039	319 (4), 177 (4), 193 (3), 205 (2), 179 (1)	164 (100), 69 (17), 149 (15), 103 (8)
	N	2032	193 (9), 319 (4), 177 (4), 205 (2), 179 (2)	164 (100), 71 (54), 81 (26), 149 (15), 103 (11)
3a	S	2234	207 (40), 179 (8), 193 (4), 435 (2)	152 (100), 120 (20), 69 (11), 109 (4), 92 (2)
	N	2224	207 (48), 193 (9), 179 (8), 165 (5), 435 (1)	152 (100), 69 (60), 120 (24), 109 (9), 81 (8)

[a] 1a, β-D-Glucoside of methyl salicylate trifluoroacetylated; 2a, eugenyl β-D-glucoside trifluoroacetylated; 3a, β-rutinoside of methyl salicylate trifluoroacetylated. [b] Retention linear index based on a series of hydrocarbons (capillary column DB5-MS).

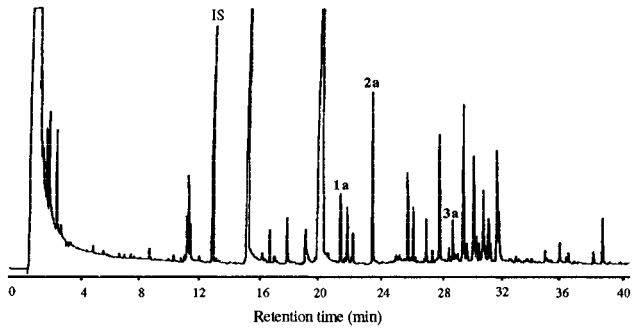

Figure 1. GC (J&W Scientific, DB5-MS) of trifluoroacetylated *P. edulis* peel extract.

previously identified (Chassagne, 1996; Chassagne et al., 1996a,b). The fragmentation rules from the EIMS of TFA glycosides (Voirin et al., 1992) allowed the spotting of peaks corresponding to glycosidically bound eugenol or methyl salicylate. As shown in Table 1, fragment ions at *m/z* 164, 149, and 103 were characteristic of eugenyl aglycon, and fragment ions at *m/z* 152, 120, and 92 were characteristic of salicylate aglycon. For the glycosidic moieties, specific fragment ions at *m/z* 193 and 319 were diagnostic for a terminal glucose and those at *m/z* 193, 207, and 435 for a terminal rhamnose. Thus, it could be postulated that glucosides and rutinosides of eugenol and methyl salicylate were present in purple passion fruit juice (Figure 2). In addition, D-glucose and L-rhamnose have been previously identified as components of the sugar moiety of passion fruit glycosides (Chassagne et al., 1995a).

By comparison, more information was obtained using NCIMS. The high electron affinity of polyhalogenated organic compounds, such as trifluoroacetylated derivatives, made this ionization mode very attractive (Budzikiewicz, 1986), and this method was used to qualitatively analyze wine and passion fruit glycosidic extracts (Chassagne et al., 1995b, 1996a). The presence of molecular M^-, adduct $[M + TFAO]^-$, and fragment $[M - TFAO]^-$ ions allowed the unequivocal determination of the molecular weight for each phenolic glycosides detected: 698, 710, and 1036 for compounds **1a**, **2a**, and **3a**, respectively. Furthermore, this ionization mode gave some information on the sugar moiety. The ions at *m/z* 547 and 885, detected in the NCIMS of the phenolic glycosides tentatively identified were the fragment ions $[GlcTFA]^-$ and $[(Rha-Glc)TFA]^-$ formed by the loss of the phenolic aglycon moiety, as shown in Figure 3 for the second one. To confirm these deductions, eugenyl and methyl salicyltate glucosides and methyl salicylate rutinoside were synthesized according to the modified Koenigs−Knorr method previously used by Baumes et al. (1989). The volatile phenolics were glycosylated with α-D-acetobromoglucose and α-acetobromorutinose and deacetylated with sodium methoxide. The ^{13}C NMR data for the synthesized glycosides and their derivatives are shown in Table 2. Except for the anomeric carbon of glucose, signals corresponding to carbohydrate moieties were similar to those observed for the corresponding known β-D-glucosides and β-rutinosides of monoterpenic alcohols (Paulsen et al., 1985; Voirin et al., 1990). Contrary to glucoside **2c** (Mulkens and Kapetanidis, 1988), NMR data for **1c** and **3c** and their acetylated derivatives have not yet been published.

The chromatographic and MS data for synthetic TFA glycosides (**1**−**3a**) and the compounds detected in natural glycosidic extracts were coincident (Table 1). Thus, the occurrence of glycosides **1c**, **2c**, and **3c** in purple passion fruit juice as well as in other passion fruit species was confirmed.

Furthermore, relative concentrations were estimated using phenyl β-D-glucopyranoside as an internal standard. The results obtained for juice and peel of four *Passiflora* commercial species, *P. edulis*, *P. edulis flavicarpa*, *P. ligularis*, and *P. mollissima*, are shown in Table 3. The phenolic glycoside levels observed in these species were in varying proportions. Only *P. edulis* contained the three glycosides in high amount. Ruti-

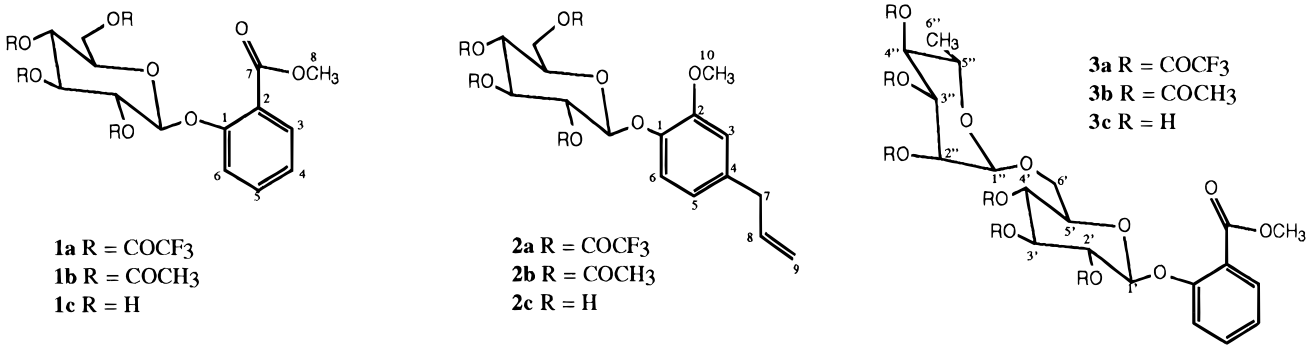

Figure 2. Chemical structures of phenolic glycosides synthesized and identified in passion fruits.

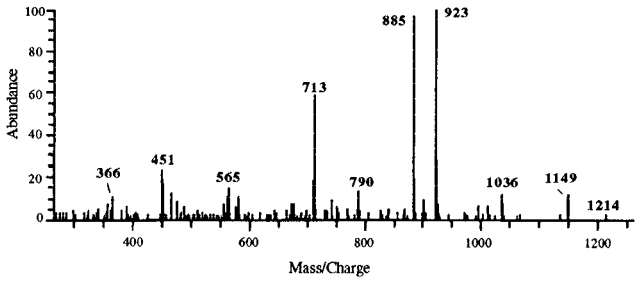

Figure 3. NCI mass spectrum of the trifluoroacetylated derivative of methyl salicylate β-rutinoside (**3a**) detected in *P. edulis* juice extract.

Table 2. ^{13}C NMR Chemical Shifts (δ) of Acetylated Glycosides 1b and 3b and Deacetylated Glycosides 1c, 2c, and 3c (62.89 MHz)

C	1b	1c	2c	3b	3c
1	155.5	156.2	144.5	155.5	156.1
2	122.5	121.7	149.5	122.0	121.9
3	131.3	131.8	114.2	131.3	131.9
4	123.0	123.9	137.3	122.9	124.0
5	133.0	135.2	122.0	133.6	135.1
6	116.9	117.5	117.4	116.3	117.6
7	166.5	166.9	39.8	166.6	169.5
8	52.1	53.6	139.0	52.1	53.6
9			116.4		
10			56.7		
1′	99.5	101.5	101.6	99.2	101.2
2′	70.9	73.7	73.7	70.7	73.7
3′	72.1	76.4	76.9	72.7	75.8
4′	68.4	70.1	70.2	69.0	70.3
5′	72.7	76.9	76.4	73.6	76.4
6′	62.0	61.3	61.3	66.6	67.3
1″				98.1	101.2
2″				69.3	71.0
3″				69.0	70.9
4″				70.8	72.8
5″				66.7	69.5
6″				17.3	17.3
glycosidic acetates					
CO	169.3−170.2		169.3−170.2		
OCH_3	20.5−20.6		20.5−20.8		

Table 3. Identification and Relative Concentrations[a] of Phenolic Glycosides in Four *Passiflora* Commercial Species

	P. edulis		*P. edulis* f *flavicarpa*		*P. ligularis*		*P. mollissima*
compd[b]	juice	peel	juice	peel	juice	peel	fruit
1c	++[c]	++	+	+	+	++	nd
2c	+	+++	+	++	+	++	++
3c	++	+	nd	nd	+	nd	nd

[a] Relative concentrations were estimated by comparison of areas of each peak with that the phenyl β-D-glucopyranoside used as internal standard (conditions of GC for trifluoroacetylated derivatives): nd, not detected; [b] **1c**, β-D-glucoside of methyl salicylate; **2c**, eugenyl β-D-glucoside; **3c**, β-rutinoside of methyl salicylate. +, <1 ppm; ++, 1-10 ppm; +++, 10-100 ppm.

noside **3c** was not found in *P. edulis flavicarpa*, and the only bound eugenyl form was detected in *P. mollissima*. Regarding the relative concentrations in the two parts of the analyzed fruits, glucosides were more abundant in the peel, particularly for *P. edulis*. This confirmed our results given above, concerning the differences in amounts of the liberated phenolic volatile compounds. Glycosidically bound eugenol has a possible role in lignin biosynthesis (Merkx and Svendsen, 1989).

While some information was available about the occurrence of eugenyl β-D-glucoside in plant tissues (Mulkens and Kapetanidis, 1988; Schwab et al., 1990; Krammer et al., 1991; Fujita et al., 1995), β-D-glucoside and β-rutinoside of methyl salicylate have never been previously reported. Nevertheless, recently, another natural glycoconjugate of methyl salicylate, a 6-*O*-(β-D-xylopyranosyl)-β-D-glucopyranoside (β-primeveroside), was identified in leaves for oolong tea (Moon et al., 1996).

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