

# Identification of Passion Fruit Glycosides by Gas Chromatography/Mass Spectrometry

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$\beta$ -D-Glucopyranosides,  $\alpha$ -L-rhamnopyranosyl  $\beta$ -D-glucopyranosides, and an  $\alpha$ -L-arabinofuranosyl  $\beta$ -D-glucopyranoside have been identified in four passion fruit species by GC/MS analysis of trifluoroacetylated (TFA) derivatives obtained from glycosidally bound volatile compounds, using NCI and/or EI ionization modes, analysis of the partially methylated alditol acetates obtained after hydrolysis of the glycosidic fraction, and comparison of the retention times of their TFA derivatives with those of synthetic standards. These compounds are quantitatively and qualitatively more important in *Passiflora edulis* and *Passiflora edulis* f. *flavicarpa* than in *Passiflora ligularis* and *Passiflora molissima*. Only (*S*)-linalool is present in bound form in *P. edulis*. (6*R*,7*E*,9*S*)-3-Oxo- $\alpha$ -ionol and 4-oxo- $\beta$ -ionol glucosides have been identified in *P. edulis* and *P. edulis* f. *flavicarpa*, and vomifoliol 1A and 2A glucosides seem characteristic of the first species.

**Keywords:** *Passion fruit; glycosidally bound compounds; gas chromatography; gas chromatography/mass spectrometry*

## INTRODUCTION

Over the past 30 years, free volatile compounds in fruits and vegetables have been extensively studied in line with the development of gas chromatography (GC) and gas chromatography coupled with mass spectrometry (GC/MS) or Fourier transform infrared (GC/FTIR) spectroscopy.

The volatiles are also present in plants as glycosidally bound components; to date, glycoconjugates have been detected in almost 170 plants belonging to 50 families (Stahl-Biskup et al., 1993; Winterhalter and Skouroumounis, 1997). Aroma compounds can be released from these nonvolatile precursors by enzymatic or chemical reactions during maturation, industrial pretreatment, or processing. As an example, vanillin, the characteristic aroma compound of vanilla, is released during the fermentation process by the action of vanilla  $\beta$ -glucosidase on vanillin glucoside (Arana, 1943).

Evidence for the presence of glycosylated conjugates in purple (*Passiflora edulis* Sims) or yellow (*Passiflora edulis* f. *flavicarpa* Degener) passion fruits has been given by the use of rapid analytical techniques (Salles et al., 1988) or from the isolation of the volatile compounds released by acid or enzymatic hydrolysis of the glycosidic extract of juice (Engel and Tressl, 1983; Winterhalter, 1990; Chassagne et al., 1995a). However, only a few studies have dealt with the structure of the bound volatile compounds in passion fruit.  $\alpha$ -L-Arabinopyranosyl  $\beta$ -D-glucopyranoside of linalool, benzyl alcohol, and 3-methylbut-2-en-1-ol have been isolated

from purple passion fruit and identified by <sup>1</sup>H NMR and MS (Chassagne et al., 1996a), and five mandelonitrile glycosides and glycosidically bound eugenol and methyl salicylate have been identified in several *Passiflora* fruits using GC/EI-MS and GC/NCI-MS of their trifluoroacetylated (TFA) derivatives (Chassagne et al., 1996b, 1997, 1998).

In this paper the on-line identification of several new edible passion fruit glycosidically bound volatile compounds by GC and GC/MS of their TFA derivatives is reported.

## EXPERIMENTAL PROCEDURES

**Reagents.** The solvents *n*-pentane, dichloromethane, and methanol were of pure grade (purity >97.7%) from Carlo Erba (Rodano, Italy) and were distilled before use.

*n*-Paraffins C<sub>8</sub>–C<sub>30</sub>, purity >95.5%, were obtained from Sigma (St. Louis, MO).

TFA reagent [*N*-methylbis(trifluoroacetamide)] was obtained from Pierce (Rockford, IL).

Amberlite XAD-2 (20–60 mesh), obtained from Röhm and Hass (Philadelphia, PA), was treated according to the procedure of Günata et al. (1985).

Hexyl and phenyl glucosides were from Sigma, vomifoliol glucosides were a gift of R. Baltenweck-Guyot and P. Albrecht, and the other glycosides used as synthetic references have been synthesized in our laboratories (Salles et al., 1990; Voirin et al., 1990; Baumes et al., 1994; Chassagne et al., 1997)

**Plant Material.** Purple passion fruits, *Passiflora edulis* Sims, from Zimbabwe, were purchased at Rungis market, France. Yellow passion fruits, *Passiflora edulis* f. *flavicarpa* Degener and *Passiflora ligularis* Juss, from Colombia, were respectively obtained from Rungis market, France, and Research Centre of the Institut de Recherche Agronomique, Njombé, Cameroun. Banana passion fruit, *Passiflora molissima* Bailey, harvested on Reunion Island, were kindly furnished by CIRAD. All fruits were at commercial maturity stage.

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**Isolation of the Glycosidically Bound Fraction.** The pulp obtained as indicated in Chassagne et al. (1997) was centrifuged (30 min, 10000g) at 4 °C, and the clear juices obtained were kept at -18 °C until analysis. Clear juice (50 mL) was poured onto a 9 × 1 cm i.d. column filled with solvent-washed XAD-2 at 1.5 mL min<sup>-1</sup>. The column was rinsed with 50 mL of distilled water, and the free volatile compounds were eluted with 50 mL of pentane/dichloromethane (2:1, v/v). The glycosidically bound fraction was then eluted using 50 mL of methanol. The eluate was dried over anhydrous sodium sulfate, filtered, and concentrated to dryness under vacuum at 45 °C. The residue constituted the crude glycosidically bound fraction (Günata et al., 1985).

**Cyanogenic Glycoside Elimination.** Passion fruit glycosidic extract was treated by 1.5 M sodium hydroxide for 4 h at room temperature (Chassagne et al., 1996b); during this treatment the corresponding glycosidic acids are produced from the passion fruit cyanogenic glycosides (Eyjolfsson, 1970). After neutralization with 1.5 M hydrochloric acid, the unhydrolyzed glycosidic compounds were recovered by fractionation on an XAD-2 column, as described above.

**Methyl Alditol Acetate Analysis.** Glycosides present in the heterosidic extract, 0.20–1 mg, were methylated as indicated by Jansson et al. (1976) by the action of methyl iodide, 0.5 mL, catalyzed by methylsulfinyl carbanion, 0.5 mL, 2 M, in dimethyl sulfoxide, 0.5 mL. The methylated compounds were then extracted with chloroform/methanol (2:1, v/v), and the extract was washed with distilled water and dried under a nitrogen current. Acid hydrolysis of the methylated glycosidically bound fraction was performed using 2 M trifluoroacetic acid (0.5 mL) at 120 °C for 1 h (Alberstein et al., 1967). The partially methylated sugars released by acid hydrolysis were converted into partially methylated alditol acetates by reduction using sodium borohydride and acetylation with acetic anhydride in the presence of perchloric acid (Harris et al., 1984). After extraction with chloroform, the organic phase was washed with distilled water and concentrated and the alditol acetates were analyzed by GC and GC/MS as indicated by Saulnier and Brillouet (1987).

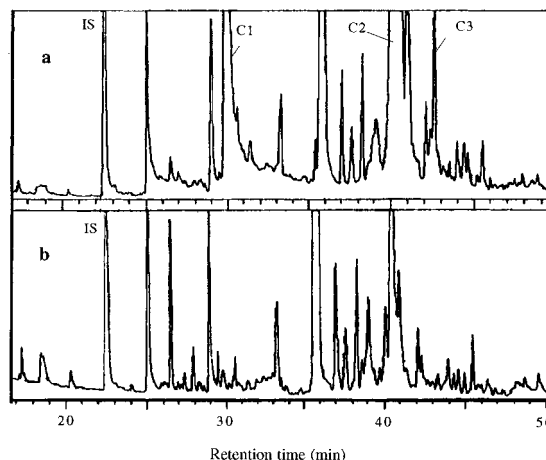
**Trifluoroacetylation.** The method described by Sweeley et al. (1963) was used. An aliquot of the methanolic solution obtained after elution of the XAD-2 column, corresponding to 500 µL of clear juice, was concentrated to dryness in a screw-capped vial at 60 °C under a stream of nitrogen. Twenty microliters of anhydrous pyridine and 20 µL of TFA reagent were added, and the vial tightly was closed, stirred, heated at 60 °C for 20 min, and then allowed to cool to room temperature.

**GC.** For TFA glycoside derivatives a Varian 3300 (Walnut Creek, CA) gas chromatograph fitted with a flame ionization detector, a DB-5 MS fused silica capillary column (30 m × 0.25 mm i.d.; 0.25 µm bonded phase; J&W Scientific, Folsom, CA), and a Shimadzu (Kyoto, Japan) C-R3A printer-plotter were used. The operating conditions were as follows: injector temperature, 280 °C; detector temperature, 300 °C; helium carrier flow rate, 1.8 mL min<sup>-1</sup>. The column temperature was raised from 125 to 220 °C at 3 °C min<sup>-1</sup> and then increased to 280 °C at 2 °C min<sup>-1</sup>.

Phenyl glucoside was used as internal standard for glycoside quantification. Three analyses, extraction and measurement, were carried out on each aglycon extract to determine the variation coefficient for each component identified.

Linear retention indices were calculated using *n*-paraffin standards (Van den Dool and Kratz, 1963).

For the alditol acetate analysis a Hewlett-Packard 5890 series II gas chromatograph fitted with a flame ionization detector and DB-1 and DB-225 capillary columns (Saulnier and Brillouet, 1987) were used. Conditions for DB-1: injection on-column; carrier gas, hydrogen (120 kPa); injector and detector temperature, 250 °C; column temperature, isothermal at 145 °C for 10 min, increased to 210 °C at 2 °C min<sup>-1</sup>. Conditions for DB-225: split injection (1/10); carrier gas, hydrogen (65 kPa); injector and detector temperature, 250 °C; column temperature, isothermal at 170 °C for 15 min, increased to 210 °C at 5 °C min<sup>-1</sup>.



**Figure 1.** Gas chromatogram of TFA passion fruit glycosidic extract before (a) and after (b) alkaline treatment at pH 12.5 for 4 h: C1, prunasin; C2, mandelonitrile  $\beta$ -rutinoside; C3, amygdalin; IS, phenyl glucoside.

Identifications were based on RRT (retention time relative to inositol, used as internal standard) on DB-1 using authentic reference compounds and confirmed by GC/MS (Bjorndal et al., 1970).

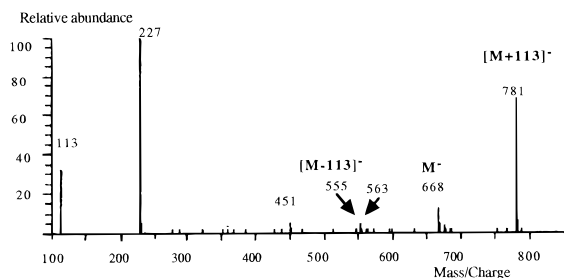
**GC/MS Analysis.** EI mass spectra were recorded by coupling an HP 5890 gas chromatograph equipped with a DB-5MS fused silica capillary column (30 m × 0.25 mm i.d.; 0.25 µm bonded phase, J&W Scientific) to an HP 5989 A mass spectrometer. Injections of ~1 µL were on-column. The transfer line was maintained at 290 °C, and the injector temperature was programmed from 110 to 260 °C at 60 °C min<sup>-1</sup> and then maintained for 55 min. The column temperature was programmed at 3 °C min<sup>-1</sup> from 125 to 290 °C. Helium at 1.1 mL min<sup>-1</sup> was the carrier gas. Source temperature was 200 °C, and mass spectra were scanned at 70 eV in the *m/z* range from 60 to 600 mass units.

NCI-MS of TFA glycosides was performed under the conditions previously described by Chassagne et al. (1995b). The operating conditions were as follows: emission current, 350 µA; energy of the electrons, 200 eV; temperatures of the source and quadrupole, 200 and 120 °C, respectively; methane was used as the reactant gas at 80 Pa, as measured at the source ion gauge. The ion source tuning was carried out in the positive ion mode by using perfluorotributylamine. Mass spectra were scanned in the range *m/z* 100–1400 at 500 ms intervals with a repeller potential of 7 V. The mass spectra reported were recorded when the abundance of pseudo-molecular ions maximized.

A DB-1 column, as indicated above, was used for the GC/MS of alditol acetates.

## RESULTS AND DISCUSSION

**Cyanogenic Compound Removal.** As shown in Figure 1a the cyanogenic glycosides previously identified as prunasin, amygdalin, and mandelonitrile rutinosides in passion fruit juices and present at high levels, 125–170 mg/kg according to the variety (Chassagne et al., 1996b), coelute with other glycosidically bound compounds in the chromatogram of the TFA crude glycosidic extract. To facilitate the identification of noncyanogenic compounds, the cyanogenic fraction removal was undertaken. At neutral pH the ionized glycosidic acids are not retained by the hydrophobic resin. The results, previously obtained by alkaline treatment at pH 12.5 for 4 h, indicate that >80% of prunasin and amygdalin was racemized and degraded to acid compounds (Chassagne et al., 1996b). Otherwise, it was checked that noncyanogenic compounds,



**Figure 2.** NCI mass spectrum of TFA 2-phenylethyl  $\beta$ -D-glucopyranoside from passion fruit.

**Table 1. Identification of Partially Methylated Alditol Acetates of Passion Fruit Glycosides**

methylated alditol acetate	RRT <sup>a</sup>	fragment ions (relative abundance)
2,3,4-tri- <i>O</i> -methylrhamnose	0.229	131 (100), 101 (95), 89 (72), 117 (70), 72 (62), 161 (25), 175 (12)
2,3,4-tri- <i>O</i> -methylarabinose	0.225	117 (100), 101 (85), 58 (40), 161 (30), 131 (20), 87 (20), 71 (20)
2,3,4,6-tetra- <i>O</i> -methylglucose	0.417	101 (100), 161 (95), 129 (65), 145 (62), 117 (62), 205 (40), 87 (30), 71 (25)
2,3,4-tri- <i>O</i> -methylglucose	0.632	101 (100), 117 (80), 129 (60), 87 (50), 161 (40), 189 (30), 233 (20)

<sup>a</sup> RRT, relative retention times, relative to inositol derivative, on DB-1 column.

octyl, heptyl, and phenyl glucosides, were not or slightly (<5%) hydrolyzed under these conditions.

The chromatogram obtained after the same alkaline treatment (Figure 1b) confirmed that the passion fruit cyanogenic glycosides had been largely removed.

**Negative Ion Chemical Ionization Mass Spectroscopy Study.** As previously reported, GC coupled to methane chemical ionization mass spectrometry provides useful information for glycoside structural determination (Chassagne et al., 1995b). The molecular mass is easily determined by the presence of the molecular ion  $M^-$  and pseudo-molecular ions  $[M - \text{TFAO}]^-$  and  $[M + \text{TFAO}]^-$ . Moreover, the mass spectra exhibited characteristic fragment ions of the sugar moiety,  $m/z$  563  $[\text{glucTFA-O}]^-$ , 677  $[\text{glucTFA-O} +$

$\text{TFAOH}]^-$  ( $-F$  is fluorine), 544  $[\text{glucTFA-O} - F]^-$ , 451  $[\text{glucTFA-O} - (-H + \text{TFAO})]^-$  for glucosides, 887- $[(\text{arab-glucTFA-O})]^-$ , 868  $[(\text{arab-glucTFA-O} - F)]^-$ , 775  $[(\text{ara-glucTFA-O} - (-H + \text{TFAO})]^-$ , 660  $[(\text{arab-glucTFA-O} - (\text{TFAO} + \text{TFAOH})]^-$ , and 437  $[\text{arabTFA-O}]^-$  for arabinosyl glucosides (the same ions are obtained for apiosyl glucosides), 901  $[\text{rutTFA-O}]^-$ , 882  $[\text{rutTFA-O} - F]^-$ , 789  $[\text{rutTFA-O} - (-H + \text{TFAO})]^-$ , 674  $[\text{rutTFA-O} - (\text{TFAO} + \text{TFAOH})]^-$ , and 451  $[\text{rutTFA-O}]^-$  for rutosides (Chassagne et al., 1995b). Preliminary information concerning the carbohydrate sequence of these glycosides can be obtained from these fragment ions.

As an example, the presence on the spectrum shown in Figure 2 of fragment ions at  $m/z$  668  $[M]^-$ , 555  $[M - \text{TFAO}]^-$ , and 781  $[M + \text{TFAO}]^-$  unequivocally indicates a mass of 668 for this TFA glycoside. Moreover, fragment ions at  $m/z$  563 and 451 suggest a glucoside structure for the carbohydrate moiety; this assumption is supported by the identification of 2,3,4,6-tetramethyl-*O*-glucose (Table 1) among the partially methylated alditol acetates obtained from passion fruit glycosidic extract. The nature of the aglycon moiety was inferred from its molecular mass, 122, calculated from the difference between the masses of the derivatized glycoside moiety (668) and the derivatized saccharidic moiety (563). A peak at  $m/z$  91 in the aglycon part of the EI-MS spectra agrees with the presence of 2-phenylethanol. From these data, the compound has been tentatively identified as 2-phenylethyl  $\beta$ -D-glucopyranoside. This assignment was confirmed by comparison of the retention time of the compound to that of a synthetic standard (Table 2).

Similarly, the presence in the spectrum shown in Figure 3 of fragment ions at  $m/z$  1038  $[M]^-$ , 925  $[M - \text{TFAO}]^-$ , and 1151  $[M + \text{TFAO}]^-$  unequivocally indicates a mass of 1038 for this TFA glycoside. Moreover, fragment ions at  $m/z$  901, 789, 674, and 451 and the identification of 2,3,4-trimethyl-*O*-rhamnose (Table 1) among the partially methylated alditol acetates obtained from passion fruit glycosidic extract suggest a rhamnopyranosyl glucoside structure for the carbohydrate moiety. The molecular mass calculated for the aglycon moiety, 154, and the EI spectra indicate the

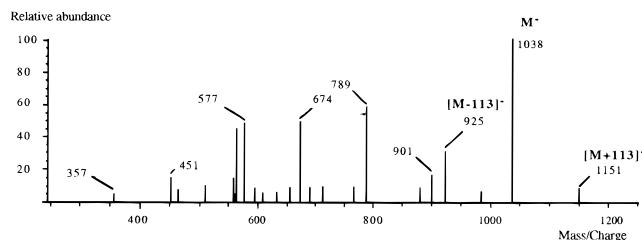
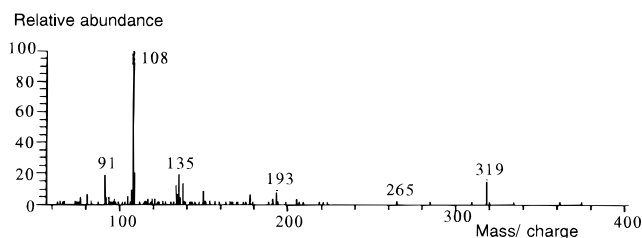
**Table 2. Monoterpene, Aliphatic, and Aromatic Alcohol Glycosides Identified or Tentatively Identified as TFA Derivatives in Several Passion Fruit Juices by GC and GC/NCI-MS**

glycoside	retention index			concentration (mg/kg)			fragment ions (relative abundance)		
	reference	unknown	<i>P. edulis</i>	<i>P. edulis</i> f <i>flavicarpa</i>	<i>P. ligularis</i>	<i>P. molissima</i>	$M^-$	$(M + 113)^-$	$(M - 113)^-$
				$\beta$ -D-Glucopyranoside					
hexanol	1640	1632	0.7 $\pm$ 0.01	0.9 $\pm$ 0.06	— <sup>a</sup>	3.8 $\pm$ 0.5	648		
benzyl alcohol	1770	1765	6.8 $\pm$ 0.4	7.6 $\pm$ 0.7	0.7 $\pm$ 0.1	0.2 $\pm$ 0.02	654 (15)	767 (100)	541 (3)
( <i>S</i> )-linalool	1818	1814	1.3 $\pm$ 0.07	0.5 $\pm$ 0.05	0.6 $\pm$ 0.1	—	700 (20)	813(100)	587 (1)
2-phenylethanol	1856	1851	2.2 $\pm$ 0.2	0.4 $\pm$ 0.1	0.5 $\pm$ 0.06	0.9 $\pm$ 0.04	668 (12)	781 (100)	555 (1)
nerol	1868	1866	tr <sup>b</sup>	—	—	0.5 $\pm$ 0.1	700 (7)	813 (100)	587 (1)
geraniol	1900	1900	0.6 $\pm$ 0.2	tr	—	tr	700 (10)	813 (100)	587 (1)
citronellol	1900	1900	0.6 $\pm$ 0.2	3.1 $\pm$ 0.6	—	—	702 (13)	815 (100)	—
methyl salicylate	1970	1963	2.6 $\pm$ 0.6	1.0 $\pm$ 0.1	0.8 $\pm$ 0.1	—	698 (8)	811 (35)	585 (1)
eugenol	2039	2032	0.8 $\pm$ 0.2	1.3 $\pm$ 0.1	0.8 $\pm$ 0.1	2.9 $\pm$ 1.0	986		
				$\alpha$ -L-Rhamnopyranosyl $\beta$ -D-Glucopyranoside (Rutinoside)					
benzyl alcohol	2118	2112	27.2 $\pm$ 3.0	17.3 $\pm$ 2.8	0.4 $\pm$ 0.02	—	992 (70)	1105 (60)	879 (100)
( <i>S</i> )-linalool	2130	2124	17.3 $\pm$ 2.8	6.3 $\pm$ 0.7	0.6 $\pm$ 0.1	—	1038 (90)	1151 (45)	925 (80)
2-phenylethanol	2191	2184	6.5 $\pm$ 0.8	5.1 $\pm$ 1.2	0.8 $\pm$ 0.1	—	1006 (25)	1119 (25)	893 (100)
geraniol	2219	2205	2.6 $\pm$ 0.1	tr	—	—	1038 (100)	1151 (20)	925 (80)
methyl salicylate	2234	2224	8.1 $\pm$ 0.6	—	0.3 $\pm$ 0.07	—	1036 (16)	1149 (11)	923 (100)
				$\alpha$ -L-Arabinofuranosyl $\beta$ -D-Glucopyranoside					
benzyl alcohol	2175	2165	3.6 $\pm$ 0.7	—	—	—	978 (25)	1091 (15)	865 (100)

<sup>a</sup> —, not detected. <sup>b</sup> tr, traces.

**Table 3. Norisoprenoid Glucosides Identified or Tentatively Identified as TFA Derivatives in Purple Passion Fruit Juice by GC and GC/EI-MS**

aglycon	retention index		fragment ions (relative abundance)	
	reference	unknown	sugar moiety	aglycon moiety
3-oxo- $\alpha$ -ionol (6 <i>R</i> ,7 <i>E</i> ,9 <i>S</i> )	2127	2140	319 (21), 193 (14), 177 (7), 205 (4), 265 (2)	108 (100), 91 (48), 135 (19), 97 (11), 81(10)
4-oxo- $\beta$ -ionol	—	2220	193 (30), 319 (14), 177 (5), 205 (3), 265 (1)	165 (100), 105 (51), 69 (19), 121 (15), 208 (14), 149 (9)
vomifoliol 1A	2215	2239	193 (3), 177 (4), 319 (16), 205 (2)	124 (100), 150 (30), 91 (7), 69 (11), 138 (5)
vomifoliol 2A	2300	2307	193 (8), 177 (5), 319 (21), 205 (7)	124 (100), 150 (40), 69 (46)

**Figure 3.** NCI mass spectrum of TFA geranyl rutinoside from passion fruit.**Figure 4.** EI mass spectrum of TFA 9-hydroxystigma-4,7-dien-3-one  $\beta$ -D-glucopyranoside (3-oxo- $\alpha$ -ionol) from passion fruit.

presence of a terpene alcohol. Geranyl  $\alpha$ -L-rhamnopyranosyl  $\beta$ -D-glucopyranoside or rutinoside was finally identified by comparison of the retention time of the compound to that of a synthetic standard (Table 2).

Monoterpene, aliphatic, and aromatic alcohol glycosides identified or tentatively identified according to this method in several passion fruit varieties are reported in Table 2. These results indicate that glycosidically bound aroma compounds are qualitatively and quantitatively more important in purple and yellow passion fruit than in the two other species studied. However, neryl glucoside, methyl salicylate rutinoside, and benzyl arabinofuranosyl glucopyranoside, as well as linalyl, benzyl, and 3-methylbut-2-en-1-yl arabinopyranosyl glucopyranosides previously identified in purple passion fruit (Chassagne et al., 1996a), are not present in the yellow passion fruit extract. Traces of 3-methylbut-2-en-1-yl arabinopyranosyl glucopyranosides are present in *P. ligularis* juice. Only glucosides have been detected in banana passion fruit (*P. molissima*).

According to Voirin et al. (1992), TFA (*R*)- and (*S*)-linalyl glycoside diastereoisomers obtained by chemical synthesis are resolved by GC. In purple passion fruit extract, comparison of the linear retention indexes with those of synthetic compounds indicates that only (*S*)-linalyl  $\beta$ -D-glucopyranoside and (*S*)-linalyl  $\alpha$ -L-rhamnopyranosyl  $\beta$ -glucopyranoside are present. (*R*)-Linalyl glucoside and (*R*)-linalyl rutinoside, previously identified in passion fruit glycosidic extract by chiral HPLC (Salles et al., 1993), were not detected under the conditions used in the present study. The chirality of the linalool released from  $\alpha$ -L-arabinopyranosyl  $\beta$ -glucopyranoside (Chassagne et al., 1996a), determined by

GC using a chiral Cyclodex-B column, was also found to correspond to the (*S*) enantiomer (D. Chassagne et al., unpublished results).

Several  $C_{13}$  norisoprenoids have been detected in the aglycon moiety released after enzymatic hydrolysis of passion fruit glycosidic extract (Winterhalter, 1990; Chassagne et al., 1995c). However, the nature and sequence of the sugars in the carbohydrate moiety are not known. As previously reported (Chassagne et al., 1995b), four peaks with low relative abundance at  $m/z$  754 and two peaks at  $m/z$  770 were detected in the reconstructed mass chromatogram. These peaks correspond to the molecular ions of the TFA derivatives of  $C_{13}$  norisoprenoid glucosides at the oxidation level of hydroxymegastigmadienone and oxygenated hydroxymegastigmadienone. The corresponding compounds were not identified due to the fact that they coeluted with much more abundant glycosidic compounds, which masked their spectra.

**Electron Impact Ionization Mass Spectroscopy Study.** EI-MS was used for the norisoprenoid glucoside identification in purple passion fruit. In the spectrum given in Figure 4, fragment ions at  $m/z$  108, 91, and 135 are characteristic of 9-hydroxymegastigma-4,7-dien-3-one (3-oxo- $\alpha$ -ionol) (Winterhalter, 1990). Moreover, fragment ions at  $m/z$  319, 193, 205, and 265 are indicative of the presence of a glucose unit (Voirin et al., 1992), and the absence of peaks characteristic of a terminal TFA deoxyhexosyl or pentosyl unit suggests 9-hydroxymegastigma-4,7-dien-3-one  $\beta$ -D-glucoside. Identification was confirmed by comparing the retention time to that of a synthetic sample synthesized by Baumes et al. (1994). GC analysis of the mixture of the four diastereoisomers obtained by these authors indicated that the retention index of the compound found in passion fruit extract (2140) is in agreement with that of (6*R*,7*E*,9*S*)-9-hydroxymegastigma-4,7-dien-3-one  $\beta$ -D-glucoside (2127). This isomer has previously been identified in raspberry (Pabst et al., 1992a) and grape (Baumes et al., 1994). The (*R*) configuration at the  $C_6$  position suggests that luteine, having the same configuration in this position, might be the precursor of this glucoside. Fragment ions characteristic of 9-hydroxymegastigma-5,7-dien-4-one (4-oxo- $\beta$ -ionol), previously identified in passion fruit juice (Winterhalter, 1990),  $m/z$  165 and 208, were detected in the spectra of one compound (Table 3). Under the conditions used, the abundance of ions characteristic of a terminal glucose unit suggests the presence of a glucoside. 9-Hydroxymegastigma-5,7-dien-4-one  $\beta$ -D-glucoside has been identified in raspberry (Pabst et al., 1992b).

It can be postulated, using the same approach, that the two compounds detected in the reconstructed chromatogram at  $m/z$  770 in NCI-MS (Chassagne et al., 1995b) were two isomers of vomifoliol glucoside. This assumption was confirmed using authentic samples isolated from Gewürztraminer wine by Baltenweck-

Guyot et al. (1996). The mass spectra and chromatographic data (Table 3) suggest the presence of vomifoliol glucosides 1A and 2A in passion fruit. Vomifoliol glucosides or roseosides are present in several plants (Bhakuni et al., 1974; Tschesche et al., 1976; Skouroumounis and Winterhalter, 1994; Strauss et al., 1987).

The norisoprenoid glucosides identified or tentatively identified using EI-MS in purple passion fruits are given Table 3. The yellow passion fruit glycosidic fraction is characterized by the absence of glycosidically bound vomifoliol (D. Chassagne, unpublished results).

The structure of several glycosides likely to release aroma compounds under chemical hydrolysis at the natural pH of fruits during processing or storage is established for the first time as edible passion fruit components.  $\beta$ -D-Glucopyranosides and  $\alpha$ -L-rhamnopyranosyl  $\beta$ -D-glucopyranosides are the major compounds except for in *P. molissima*, in which only  $\beta$ -D-glucopyranosides have been detected. The results obtained show a great qualitative and quantitative variability among the varieties studied, the glycosidically bound compounds being more important in *P. edulis* than in *P. edulis* f. *flavicarpia*, whereas only minute quantities of these compounds have been detected in *P. ligularis* and *P. molissima*.

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