Identification and Quantification of Passion Fruit Cyanogenic Glycosides

David Chassagne,*,† Jean C. 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 Substances Naturelles, Institut des Produits de la Vigne, INRA, 2 Place Viala, 34060 Montpellier Cedex 01, France

Five mandelonitrile glycosides have been detected in the glycosidic fraction isolated from several *Passiflora* fruits using GC/EI-MS or GC/NCI-MS of trifluoroacetylated derivatives. Reasons for the possible co-occurence of prunasin and sambunigrin in passion fruit juice and peel are given, and amygdalin is reported for the first time as a passion fruit component. Two mandelonitrile rhamnopyranosyl β-D-glucopyranosides have been tentatively identified by MS. The extraction of cyanoglycosides using Amberlite XAD-2 followed by GC analysis of TFA derivatives was found to be an efficient method for their rapid determination. Prunasin was found to be the most important cyanogenic glycoside in peel (285 mg/kg for *P. edulis* f. *flavicarpa*), whereas amygdalin (31 mg/kg for *P. edulis*) and the two compounds tentatively identified as mandelonitrile rhamnopyranosyl β-D-glucopyranosides were mostly found in the juice (99 mg/kg for *P. edulis* f. *flavicarpa).* Different amounts of sambunigrin were found in the juice and the peel (from 0.4 mg/kg in *P. edulis* juice to 15.5 mg/kg in *P. edulis* f. *flavicarpa* peel).

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

INTRODUCTION

Cyanogenesis, the ability of plants and other organisms to liberate hydrogen cyanide, has been detected in 3000 plant species from 110 botanical families (Poulton, 1990).

Hydrogen cyanide in plants is generally released from cyanogenic glycosides, glycosidic derivatives of α-hydroxynitriles. Most of these are simple glucosides, such as linamarin, dhurrin, or prunasin, although disaccharidic and trisaccharidic derivatives have been identified: amygdalin, vicianin, and xeranthin.

These compounds also differ according to the nature of the aglycon moieties derived from aromatic, aliphatic, or cyclopentenoid amino acids.

Approximately 75 cyanogenic glycosides have been identified in plants, including food plants such a cassava, lima bean, sorghum, and members of Passifloraceae.

Passiflora edulis was reported to be cyanogenic as early as 1919 (Rosenthaler, 1919). However, prunasin [2(*R*)-(β-D-glucopyranosyl)-2-phenylacetonitrile] was only identified in *P. edulis* SIM (purple passion fruit) leaves and fruit and *P. edulis* f. *flavicarpa* (yellow passion fruit) (Spencer and Seigler, 1983).

Several analytical methods have been developed for studying these compounds. Hydrogen cyanide and glucose released by enzymatic hydrolysis can be determined according the methods of Lambert (1975). More recently, HPLC was used to directly determine cyanogenic glycosides (Cairns et al., 1978; Schilder and Wilkens-Sauer, 1986; Stochmal and Oleszek, 1994).

GC can be advantageously coupled to a very sensitive detector, especially a mass spectrometer (Cairns et al., 1978). In this case, precolumn derivatization, trimethylsilylation, or trifluoroacetylation is required.

In a previous work Chassagne et al. (1995a) detected several glycosidic compounds characterized by the presence of an aglycon moiety identified as 2-hydroxy-2-phenylacetonitrile or mandelonitrile, after enzymatic hydrolysis of purple passion fruit glycosidic extract. It was, however, only possible to identify prunasin, previously reported by Spencer and Seigler (1983).

The objective of the present work is to identify by GC/MS the trifluoroacetylated derivatives of purple passion fruit glycosidic compounds. GC/MS has been successfully used for grape (Voirin et al., 1992a,b) and passion fruit (Chassagne et al., 1995a) glycosidic compound identification. Another objective is the use of this method to quantify trifluoroacetylated derivatives of glycosidic compounds in several edible *Passiflora* species: *P. edulis* SIM, *P. edulis* f. *flavicarpa, P. ligularis,* and *P. mollissima*

MATERIALS AND METHODS

Reagents and Reference Samples. Analytical reagent grade solvents were further purified by distillation before use. Amberlite XAD-2 resin from Röhm and Haas was purified according to the procedure of Günata et al. (1985). Trifluoroacetylating reagent *N*-methylbis(trifluoroacetamide) from Pierce and phenyl β-D-glucopyranoside from Sigma were used.

Prunasin and amygdalin were obtained from Extrasynthese (Genay, France).

Plant Material. *P. edulis* SIM (purple passion fruits) from Zimbabwe and *P. ligularis* from Colombia were purchased at Rungis market. *P. edulis* f. *flavicarpa* (yellow passion fruits) were grown in the Centre de Recherches IRA Nyombé (Cameroon), and the *P. mollissima* grown in La Réunion were supplied by CIRAD.

Passion fruits (with the exception of *P. mollissima*) were cut and the seeds removed by filtration through gauze. The pulp was centrifuged (30 min, 10000*g*) at 4 °C. *P. mollissima*

* Author to whom correspondance should be addressed (e-mail Chassagne@gbsa.arpb.univ-montp2.fr).

† Laboratoire de Génie Biologique et Sciences des Aliments.

‡ Laboratoire des Arômes et Substances Naturelles.

S0021-8561(96)00381-0 CCC: $12.00

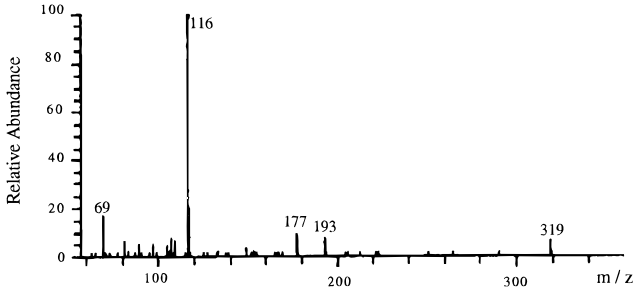

Figure 1. GC/EI-MS of trifluoroacetylated amygdalin in passion fruit glycosidic extract.

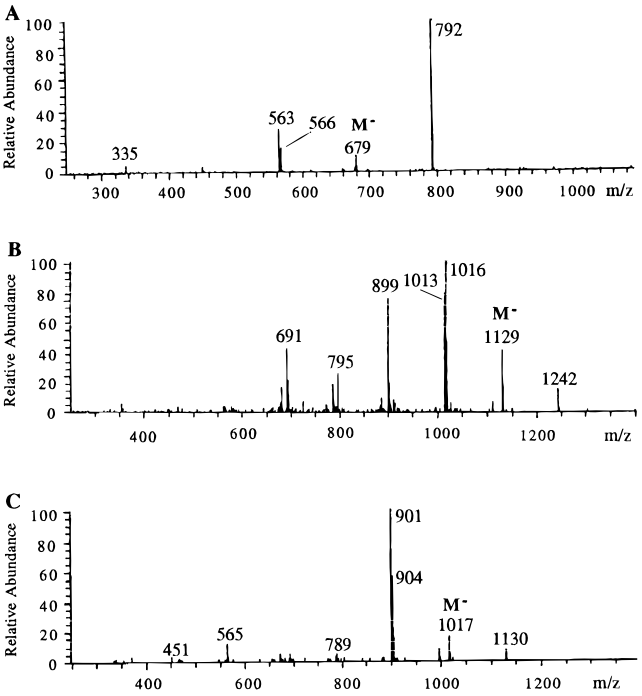

Figure 2. GC/NCI-MS of (A) trifluoroacetylated prunasin, (B) amygdalin, and (C) mandelonitrile rhamnopyranosyl β-D-glucopyranoside in passion fruit glycosidic extract.

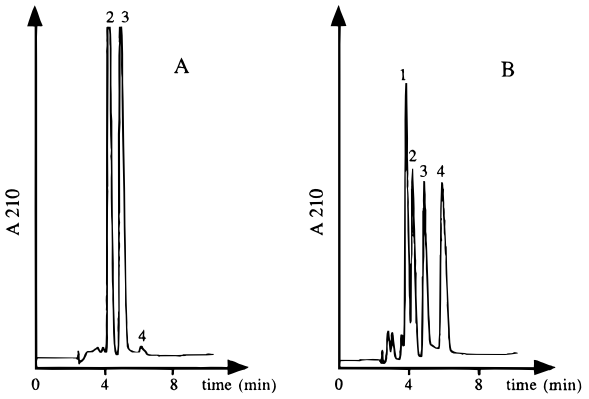

Figure 3. HPLC chromatograms of a mixture of mandelonitrile glycosides (A) before and (B) after basic treatment for 1 h at pH 11 and 25 °C . A Cyclobond I column, 4.6 × 250 mm, was used with mobile phase acetonitrile−water (1:99 v/v) at 1.5 mL min^{-1}; detection was at 210 nm. Peaks: 1, neoamygdalin; 2, amygdalin; 3, prunasin; 4, sambunigrin.

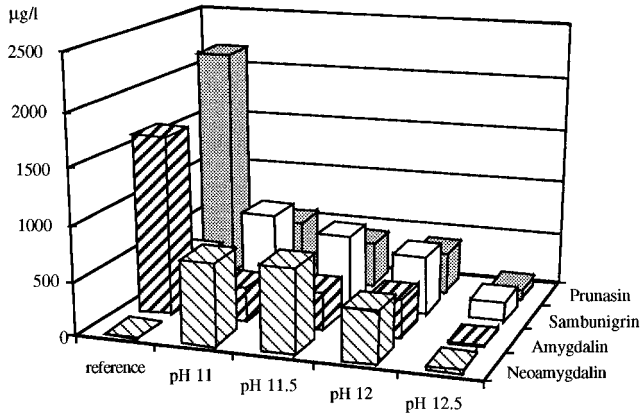

Figure 4. Isomerization of amygdalin and prunasin during basic treatment at pH 11, 11.5, 12, and 12.5 for 4 h at 25 °C. Glycoside concentrations were determined by GC of trifluoroacetylated derivatives, DB-5MS fused silica capillary column, 30 m × 0.25 mm i.d., 0.25 μm bonded phase.

was sliced and crushed in a Waring blender for 2 min in the presence of deionized water (1:1 w/v). The homogenate obtained was centrifuged in the conditions indicated above.

The clear juices obtained were kept at −18 °C until analysis. Passion fruit peel was cut into small pieces and crushed in a Waring blender for 2 min in the presence of methanol (1:2 w/v). After 20 min of contact, the mixture was filtered under vacuum. The methanol was evaporated under vacuum at 45 °C and the residue dissolved in deionized water.

Isolation of Glycosidic Extract. Passion fruit juice was fractionated on XAD-2 resin as described by Günata et al. (1985). A 50-mL volume of pentane−dichloromethane (2:1 v/v) was used to elute free aroma fractions, which were then discarded. A 50-mL volume of methanol was used to elute the bound fraction. The bound fractions were dried over anhydrous sodium sulfate.

Trifluoroacetylation. The bound fraction obtained from 0.5 mL of passion fruit juice was concentrated to dryness in a small screw-capped vial at 60 °C under nitrogen and derivatized according to the method of Voirin et al. (1992a). Phenyl β-D-glucopyranoside (10 μg) was used as internal standard.

Treatment in Basic Medium. Amygdalin and prunasin water solutions (1.5−5 mg/L) at pH 11, 11.5, 12, and 12.5 in the presence of 1.5 M sodium hydroxide were treated for 1 and 4 h at 25 °C. The medium was neutralized using 1.5 M hydrochloric acid, and the compounds were trifluoracetylated for GC or used without modification for chiral HPLC.

HPLC. A Shimadzu LC 9A (Kyoto, Japan) pump system was used, fitted with a Cyclobond I column, 4.6 × 250 mm (Astec, Whippany, NJ), a Rheodyne (Cotati, CA) 7125 sampling valve, a Varian 2550 UV detector (Walnut Creek, CA) operated at 210 nm, and a Shimadzu CR 6A detector. The column was eluted with acetonitrile−water (1:99 v/v) at a flow rate of 1.5 mL/min

GC/MS Analysis. Mass spectra were recorded for TFA derivatives by coupling a HP 5890 gas chromatograph equipped with a DB-5MS fused silica capillary column (30 m × 0.25 mn i.d.; 0.25 μm bonded phase; J&W Scientific, Folsom, CA) to a HP 5889 A mass spectrometer.

Injections of about 1 mL were on column; the injector temperature was programmed at 3 °C/min from 125 to 290 °C with helium as carrier gas at 1.1 mL/min. The transfer line was heated at 290 °C, and the injector temperature was programmed from 110 to 260 °C at 6 °C/min and then maintained for 50 min.

For EI-MS. Source temperature was 200 °C; mass spectra were scanned at 70 eV in the range *m*/*z* 60−600 uma.

For NCI-MS. Operating conditions were as follows: emission current, 350 mA; energy of the electrons, 200 eV. The temperatures of the source and quadrupole were 200 and 120 °C, respectively; methane was used as the reactant gas at 80 Pa, as measured at the source ion gauge. 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. Mass spectra were recorded when the abundance of pseudomolecular ions was at maximum.

Table 1. Retention and Electronic Impact Mass Spectrometric Data of Passion Fruit Trifluoroacetylated Cyanogenic Glycosides

glycoside	RI[a] ref	RI[a] unknown	EI mass spectrometric data: saccharidic moiety	EI mass spectrometric data: aglycon moiety
(*R*)-Mand[b] Glc*p* (**A**) (prunasin)	1921	1919	193 (14), 310 (3), 177 (1), 165 (1)	116 (100), 69 (19), 89 (11), 133 (4)
(*S*)-Mand Glc*p* (**B**) (sambunigrin)	1890	1883	319 (19), 193 (17), 177 (9), 205 (5)	116 (100), 69 (22), 89 (10), 133 (2)
(*R*)-Mand Glc*p*-Glc*p* (**C**) (amygdalin)	2328	2330	319 (18), 193 (14), 177 (14), 205 (2)	116 (100), 69 (33), 89 (6), 133 (1)
Mand Rha*p*-Glc*p*[c] (**D**) (isomer 1)		2251	207 (31), 193 (9), 179 (7), 292 (4), 279 (2), 435 (2)	116 (100), 69 (14), 89 (5), 133 (3)
Mand Rha*p*-Glc*p*[c] (**E**) (isomer 2)		2263	207 (60), 193 (23), 179 (3), 435 (3), 278 (1), 292 (1)	116 (100), 69 (13), 89 (6)

[a] Linear retention index, DB-5MS fused capillary column (30 m × 0.25 mm i.d., 0.25 μm bonded phase). [b] Mand, madelonitrile. [c] Tentatively identified.

Table 2. Cyanogenic Glycoside Content (Milligrams per Kilogram) of Juice and Peel of Several Cultivated *Passiflora* Species

glycoside	*P. edulis* juice	*P. edulis* peel	*P. edulis* f. *flavicarpa* juice	*P. edulis* f. *flavicarpa* peel	*P. ligularis* juice	*P. ligularis* peel	*P. mollissima* whole fruit
sambunigrin	0.4 ± 0.1	5.6 ± 1.0	3.2 ± 0.6	15.7 ± 0.2	nd[a]	nd	nd
prunasin	43.1 ± 5.0	231.4 ± 5.0	56.4 ± 4.5	286.9 ± 8.8	nd	1.2 ± 0.1	0.7 ± 0.3
mandelonitrile rutinoside (1)[c]	40.4 ± 0.5	17.7 ± 3.4	99.6 ± 3.4	62.1 ± 2.5	nd	tr[b]	nd
mandelonitrile rutinoside (2)[c]	10.4 ± 0.8	11.4 ± 1.1	nd	nd	nd	nd	nd
amygdalin	31.3 ± 1.2	19.6 ± 3.3	14.4 ± 0.6	1.4 ± 0.2	nd	nd	nd
HCN[d] (μmol/g)	0.328 ± 0.023	0.910 ± 0.037	0.567 ± 0.026	1.168 ± 0.036	nd	0.004	0.002 ± 0.001

[a] nd, not detected. [b] tr, trace. [c] Tentatively identified. [d] Calculated amount of HCN equivalence that could be released with enzymic preparation.

RESULTS AND DISCUSSION

EI mass spectra of TFA derivatives previously detected as mandelonitrile glycosides (Chassagne et al., 1995a) are given in Table 1. These spectra are very similar for aglycon moieties, although the presence of a parent ion at *m*/*z* 116 (φ-$CH_2CN)^+$ clearly indicates the presence of mandelonitrile as, for example, for amygdalin (Figure 1).

Fragment ions of less important intensity at *m*/*z* 319, 193, and 205 indicate the presence of glucose in saccharidic moieties of compounds **A**, **B**, and **C**, whereas low-intensity fragment ions at *m*/*z* 435, 207, 193, 292, and 179 suggest the presence of a rhamnose−glucose unit for the compounds **D** and **E** (Voirin et al., 1992a).

NCI mass spectra (Chassagne et al., 1995b) are different from the above, being characterized by the presence of a molecular ion M^-, an adduct *m*/*z* $(M + TFAO)^- = (M + 113)^-$, and an ion at *m*/*z* $(M - TFAO)^- = (M - 113)^-$ (Figure 2).

What is more, ions characteristic of the saccharidic moiety were detected at *m*/*z* 563 (Glc*p*-TFAO)$^-$ for compounds **A** and **B**, at *m*/*z* 1013 (Glc*p*-Glc*p*-TFAO)$^-$ for compound **C**, and at *m*/*z* 901 (Rha*p*-Glc*p*-TFAO)$^-$ for compounds **D** and **E**.

From these results, the presence of two mandelonitrile glucopyranosides (**A** and **B**), one mandelonitrile gentiobioside (**C**), and two mandelonitrile rhamnopyranosyl glucopyranosides (**D** and **E**) can be postulated. The presence of a gentiobioside moiety in passion fruit heterosidic extract has been previously established (Chassagne et al., 1995c).

Reference compounds were used by GC to identify prunasin (**A**) and amygdalin (**C**). Sambunigrin (**B**) has been shown to be present in a sample obtained after racemization of prunasin for 1 h at pH 11 and from trace amounts of this compound in the prunasin reference compound.

Two hypotheses can be formulated concerning the detection of sambunigrin (**B**) in passion fruit glycosidic extract. The first is that this compound is a natural compound of passion fruit. The co-occurence of the two epimers, prunasin and sambunigrin, is well established, these two compounds having been found in different ratios in *Accacia*, subgenus *Phylladineae* (Maslin et al., 1988), and *Aculeiferum* (Conn et al., 1989).

The second hypothesis is that sambunigrin can result from the epimerization reaction that occurs during the derivatization process; Nahrstedt (1975) reported the occurrence of mandelonitrile epimerization in basic medium, the basic solvent pyridine having been used during TFA derivatization. This hypothesis can, however, be discarded according to the results obtained during TFA derivatization of a pure sample of amygdalin, when neoamygdalin was not detected. On the contrary, compounds identified as *S* isomers, neoamygdalin and sambunigrin, by chiral HPLC (Salles et al., 1993) (Figure 3) and GC of trifluoroacetyl derivatives (Figure 4) were produced during treatment of *R* isomers in high basic medium. In these conditions alkaline hydrolysis of cyanogenic compounds occurs. These results, together with the resemblance of their EI mass spectra, suggest that the two tentatively identified mandelonitrile rhamnopyranosyl β-D-glucopyranosides (**D** and **E**) are epimeric forms of the same compound (Figure 5). To the best of our knowledge, these structures have not been previously reported.

Cairns et al. (1978) have previously reported the efficiency of GC for the identification and quantitative determination of TFA *R* and *S* amygdalin epimers. Recovery yields of pure cyanoglycosides (prunasin and

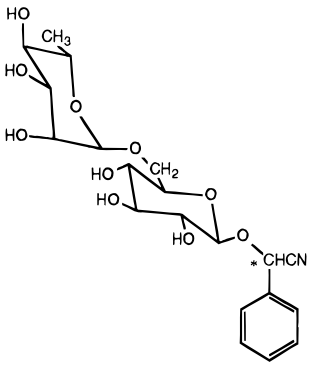

Figure 5. Mandelonitrile rhamnopyranosyl β-D-glucopyranoside tentatively identified in passion fruit glycosidic extract.

amygdalin) after fixation of these compounds on Amberlite XAD-2 resin and elution with methanol were found to be equal to 99 and 89%, respectively.

This led us to conclude that the extraction of cyanoglycosides using Amberlite XAD-2 followed by GC/MS analysis of TFA derivatives is an efficient method for rapidly determining plant cyanogenic glycosides.

The results obtained concerning the juice and peel content of four *Passiflora* species, *P. edulis* SIM, *P. edulis* f. *flavicarpa*, P. *edulis ligularis*, and *P. mollissima*, are given in Table 2. Low levels of cyanogenesis were detected in *P. ligularis* and *P. mollissima*, and prunasin was the only cyanogenic compound present in the peel of the first species.

Large quantities of cyanogenic compounds are normally found in *P. edulis* SIM and *P. edulis* f. *flavicarpa*, particularly in the peel. The most important compound present in this part of the fruit is prunasin, representing 80% of cyanogenic glycosides. The quantity of prunasin detected in juice is about 5-fold less than that found in peel, with small quantities of sambunigrin being present in the juice and peel. The fact that the ratio between these two epimers varies according to the part of the fruit and the nature of the species analyzed indicates that sambunigrin is not produced by epimerization of prunasin during the derivatization reaction and is therefore a natural product.

The content of amygdalin and of the two isomeric compounds tentatively identified as mandelonitrile rhamnopyranosyl β-D-glucopyranoside is higher in juice than in peel.

The cyanhydric acid concentration calculated from these data (Table 2) varies from 0.33 (purple passion fruit juice) to 1.17 mmol/g of fresh matter (yellow passion fruit juice). These concentrations are 5-fold less than those reported by Seigler and Spencer (1983), possibly due to differences in fruit maturity and origin.

LITERATURE CITED

Cairns, T.; Froberg, J. E.; Gonzales, S.; Langham, W. S.; Stamp, J. J.; Howie, J. K.; Sawyer, D. T. Analytical chemistry of amygdalin. *Anal. Chem.* **1978**, *50*, 317−322.

Chassagne, D.; Crouzet, J. Free and bound volatile of temperate and tropical fruits. In *Fruit Flavors. Biogenesis, Characterization and Authentification*; Rouseff, R. L., Leahy, M. M., Eds.; American Chemical Society: Washington, DC, 1995a; pp 182−189.

Chassagne, D.; Crouzet, J.; Baumes, R. L.; Lepoutre, J. P.; Bayonove, C. L. Determination of trifluoroacetylated glycosides by gas chromatography coupled to methane negative chemical ionization mass spectrometry. *J. Chromatogr. A* **1995b**, *694*, 441−451.

Chassagne, D.; Bayonove, C.; Crouzet, J.; Baumes, L. R. Formation of aroma by enzymic hydrolysis of glycosidically bound components of passion fruit. In *Bioflavour '95, Analysis, Precursors Studies, Biotechnology*; Etievant, P., Schreier, P., Eds.; INRA: Paris, 1995c; pp 217−222.

Conn, E. E.; Seigler, D. S.; Maslin, B. R.; Dunn, J. Cyanogenesis in *Acacia* subgenus *Aculeiferum*. *Phytochemistry* **1989**, *28*, 817−820.

Günata, Y. Z.; Bayonove, C.; Baumes, L. R.; Cordonnier, R. The aroma of grapes: extraction and determination of free and glucosidically bound fractions of some grape aroma components. *J. Chromatogr.* **1985**, *331*, 83−90.

Lambert, J. L.; Ramasamy, J.; Paukstelis, J. V. Stable reagents for the colorimetric determination of cyanide by modified König reactions. *Anal. Chem.* **1975**, *47*, 916−918.

Maslin, B. R.; Dunn, J. E.; Conn, E. E. Cyanogenesis in australian species of *Acacia*. *Phytochemistry* **1988**, *27*, 421−428.

Nahrstedt, A. Die Isomerisierung von Amygdalin und Homologen. *Arch. Pharm.* **1975**, *308*, 903−910.

Poulton, J. E. Cyanogenesis in plants. *Plant Physiol.* **1990**, *94*, 401−405.

Rosenthaler, L. Distribution of hydrocyanic acid in plant kingdom. *Schweiz. Apoth. Ztg.* **1919**, *57*, 279−283, 295−297, 307−313, 324−329, 341−346.

Salles, C.; Jallageas, J. C.; Crouzet, J. C. HPLC separation of fruit diastereoisomeric monoterpenyl glycosides. *J. Essent. Oil Res.* **1993**, *5*, 381−390.

Schilder, V. H.; Wilkens-Sauer, M. Quantitative Bestimmung Cyanogener Glykoside in *Linum usitatissimum* mit Hilfe der HPLC. *Fette, Seifen, Anstrichm.* **1986**, *8*, 287−290.

Spencer, K. C.; Seigler, D. S. Cyanogenesis of *Passiflora edulis*. *J. Agric. Food Chem.* **1983**, *31*, 794−796.

Stochmal, A.; Oleszek, W. Determination of cyanogenic glucosides in white clover (*Trifolium repens L.*) by high performance liquid chromatography. *Phytochem. Anal.* **1994**, *5*, 271−272.

Voirin, S. G.; Baumes, R.; Günata, Y. Z.; Bitteur, S.; Bayonove, C. L. Analytical methods for monoterpene glycosides in grape and wine. I. XAD-2 extraction and gas chromatographic-mass spectrometric determination of synthetic glycosides. *J. Chromatogr.* **1992a**, *590*, 313−328.

Voirin, S. G.; Baumes, R.; Sapis, J. C.; Bayonove, C. L. Analytical methods for monoterpene glycosides in grape and wine. II. Qualitative and quantitative determination of monoterpene glucosides in grape. *J. Chromatogr.* **1992b**, *595*, 269−281.

Received for review April 16, 1996. Revised manuscript received September 25, 1996. Accepted October 8, 1996.[⊗]

JF960381T

[⊗] Abstract published in *Advance ACS Abstracts*, November 15, 1996.