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Determination of trifluoroacetylated glycosides by gas chromatography coupled to methane negative chemical ionization mass spectrometry

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Abstract

Gas chromatography coupled to methane chemical ionization mass spectrometry was used for qualitative determination of trifluoroacetylated plant glycosides. The mass spectra obtained exhibited characteristic fragment ions of the sugar moiety and molecular or pseudo-molecular ions. The pattern of these chemical ionization spectra was influenced by source temperature. This method was applied to the structural determination of glycosides from purple passion fruit and muscat wine (Muscat of Frontignan) extracts obtained by adsorption on Amberlite XAD-2.

1. Introduction

The occurrence of glycosidically bound volatile components such as monoterpenic, aromatic and aliphatic alcohols, phenols, C-13 norisoprenoids or polyols in fruits such as grape [1,2], papaya [3] and passion fruit [4,5] has been reported. Several methods for the extraction and determination of these non-volatile components have been described, but their qualitative determination involved time-consuming and laborious steps [1,6–8]. Recently, a method for the direct analysis of glycosidic extracts by gas chromatography (GC) coupled to mass spectrometry (MS) after derivatization was reported [9,10]. Trifluoroacetylated (TFA) derivatization proved most suitable for qualitative and quantitative analysis of monoterpenyl glycosides in electron impact (EI) MS, but chemical ionization (CI) in the positive ion mode gave unsatisfactory results, except for some terpenyl and aryl monoglucosides. The high electron affinity of polyhalogenated organic compounds, as the TFA derivatives, made negative ion CI-MS (NCI-MS) very attractive to analyse directly TFA glycosides [11]. Thus, this paper reports the GC–NCI-MS analysis of TFA derivatives of glycosides previously synthesized [12], and its application to the qualitative analysis of glycosides from passion fruit and muscat wine extracts.

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2. Experimental

2.1. 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. [6]. The trifluoroacetylating reagent N-methylbis-(trifluoroacetamide), phenyl and octyl β-D-glucopyranoside were obtained from Sigma. Standard glycosides were synthesized as reported [12].

2.2. Plant material

Mature purple passion fruits (*Passiflora edulis* Sims) from Zimbabwe were purchased in France (Rungis) and stored at 4°C until processing. The wine was made from Muscat of Frontignan grapes grown at the vineyard of the wine experimental station in Pech-Rouge (South of France) by standard wine processing.

2.3. Isolation of natural glycosidic components

Passion fruit was cut, and the seeds were removed by filtration through a gaze. The juice and the pulp were centrifuged (30 min, 10 000 *g*) at 4°C. The clear juice obtained was kept at −18°C until analysis. Muscat wine and passion fruit juice were fractionated on XAD-2 resin as described by Günata et al. [6]. A 50-ml volume of pentane–dichloromethane (2:1, v/v) was used for the elution of free aroma fractions and those were discarded. A 50-ml volume of methanol was used for the elution of the bound fraction from passion fruit whereas this fraction was obtained using 50 ml of ethyl acetate in the case of muscat wine. The bound fractions were dried over anhydrous sodium sulfate.

2.4. Trifluoroacetylation

The bound fraction obtained from 1 ml of passion fruit juice, 20 ml of muscat wine or a mixture of synthetic glycosides 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. [9].

2.5. Direct GC–NCI-MS analysis of the bound fractions

NCI-MS spectra were recorded for the TFA derivatives by coupling a Hewlett-Packard (HP) 5890 gas chromatography equipped with a DB-5 fused-silica capillary column (30 m × 0.32 mm I.D.; 0.25 μm bonded phase; J & W Scientific), to a HP 5889A mass spectrometer. The transfer line was heated at 290°C. Injections of about 1 μl were on column: the injector temperature was programmed at 60°C/min from 110 to 260°C 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.

For NCI-MS, the operating conditions were as follow; emission current: 350 μA; 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. 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.

3. Results and discussion

In a first step, the TFA derivatives of commercial or synthesized glycosides were studied by GC–NCI-MS with methane as moderating gas. Methane was chosen because it is the most popular CI reagent: as the obtained results were satisfactory, no other gas was investigated.

3.1. Capillary GC–NCI-MS

A representative reconstructed total ion current (TIC) chromatogram from the GC–NCI-MS analysis of standard glycosides is shown in Fig. 1.

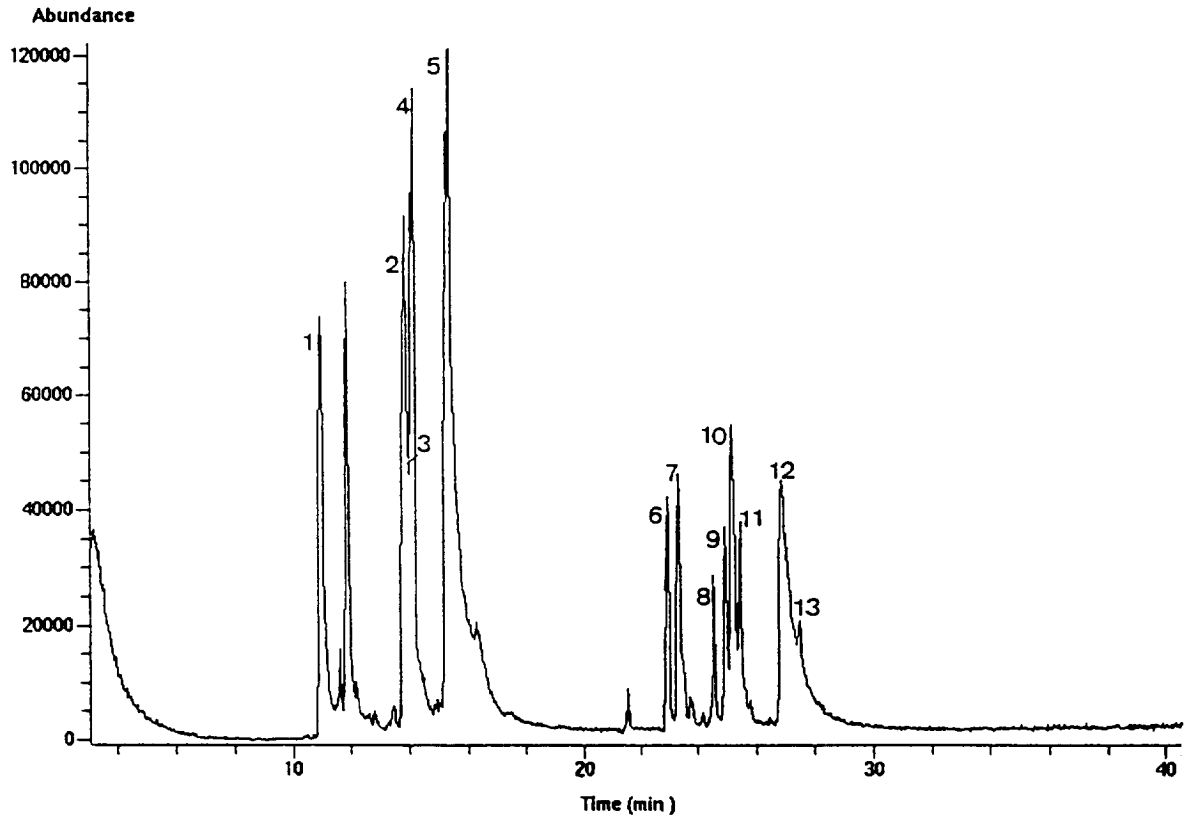

Fig. 1. Total ion chromatogram of TFA derivatives of standard glycosides. This sample was analysed by using methane negative ionization. Peaks: 1 = phenyl, 2 = octyl, 3 = (*R*)-linalyl, 4 = (*S*)-linalyl, 5 = 2-phenylethyl glucosides; 6 = (*R*)-linalyl, 7 = (*S*)-linalyl, 10 = 2-phenylethyl rutinosides; 8 = (*R*)-linalyl, 9 = (*S*)-linalyl, 12 = 2-phenylethyl arabinosylglucosides; 11 = linalyl, 13 = 2-phenylethyl apiosylglucosides.

GC–NCI-MS displayed the same degree of resolution of glycosides as that reported under EI analytical conditions by Voirin et al. [9] for the same mixture, allowing easy comparison of the obtained chromatograms.

3.2. Characteristic fragment ions

The operational parameters (see below) were determined to obtain high relative abundance of analyte specific ions which might give structural information for both glucosides and diglycosides studied. The NCI-MS spectra of TFA derivatives of linalyl β-D-glucopyranoside and β-rutinoside are shown in Fig. 2 as characteristic examples. The two most abundant ions of low mass in these spectra were characteristic of the derivatizing group (TFA): These ions at *m/z* 113 and 227 would correspond to $[TFAO]^-$ and $[2TFAOH - H]^-$ ions, respectively, formed by dissociative electron capture of the sample [13].

However, spectra obtained in the negative ion mode are generally characterized by the presence of ions specific of the analyte, i.e. molecular or pseudo-molecular ions [11]. Under the experimental conditions used, molecular ions M^- for glycosides were observed, pointing out an ionization by electron-capture reaction. The TFA group is known to increase the electron-capture probability, either in GC–electron-capture detection (ECD) or GC–NCI-MS, as already observed for the TFA derivatives of chlorophenols and chloroanilines with methane as moderating gas [13]. For all the glycosides

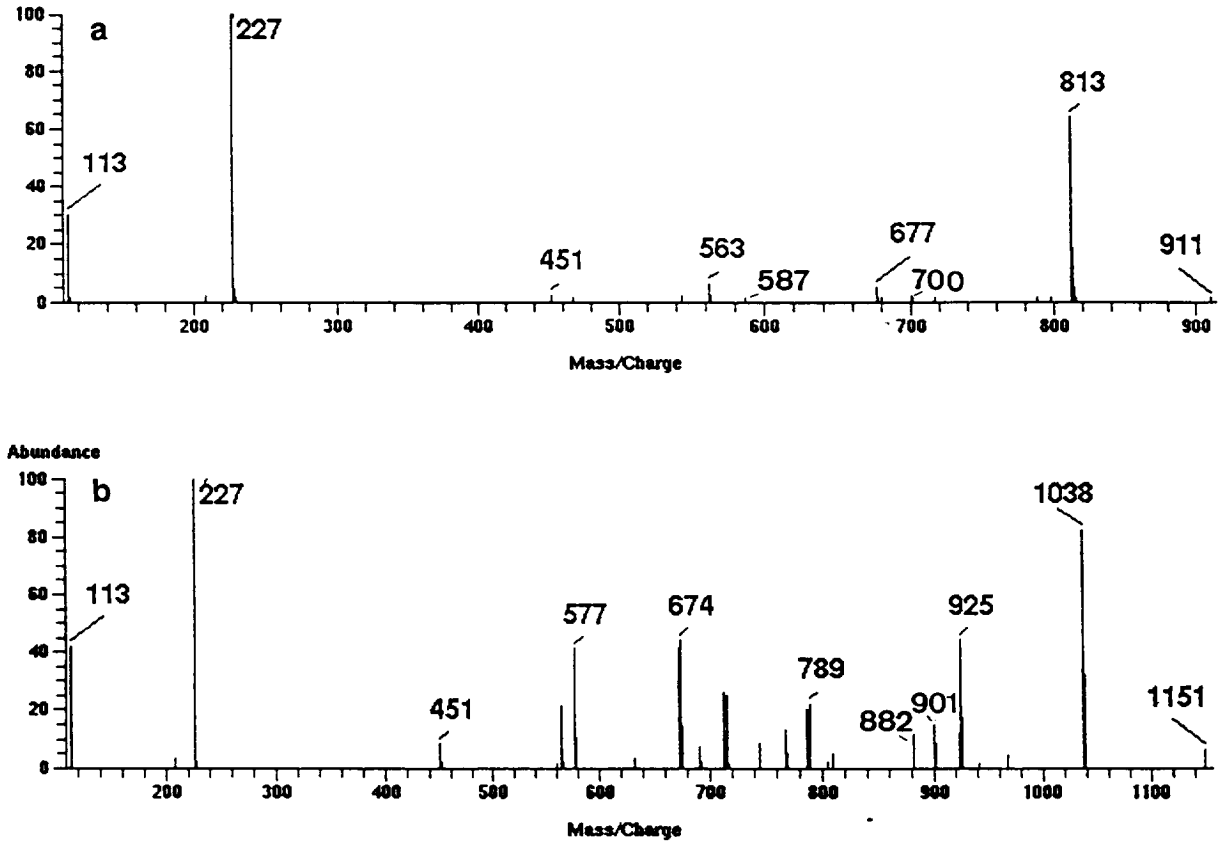

Fig. 2. NCI-MS spectra of TFA derivatives of (a) linalyl glucopyranoside and (b) linalyl β-D-rutinoside.

studied, an adduct ion $[M + TFAO]^-$ was observed at an m/z value corresponding to [M + 113] with much higher abundance for glucosides than for diglycosides. This adduct ion would be formed by TFAO, produced by dissociative electron capture of the sample, which would associate with additional sample [14]. No other artifacts such as alkylated adduct ions, sometimes observed when CH_4 was used as the moderating gas, were observed [15]. Moreover a fragment ion $[M - TFAO]^-$ was obtained by dissociative electron capture of each glycoside studied. Thus, the presence of the specific ions M^-, $[M - TFAO]^-$ and $[M + TFAO]^-$ in our conditions allowed the unequivocal determination of the molecular mass for each glycoside (Table 1).

On the other hand, NCI-MS of glycosides gave some information concerning the sugar moiety. Indeed, the ions at m/z 563, 887 and 901 are fragment ions specific of TFA sugar units corresponding respectively to $[GlcTFA \sim O]^-$, $[AraTFA \sim GlcTFA \sim O]^-$ and $[RutTFA \sim O]^-$ ions (where ~ indicates that a bond is present),

Table 1
Molecular, adduct and fragment ions observed in NCI-MS spectra of TFA derivatives of glycosidic standards

Derivatives (peaks in Fig. 1)	M^-	$[M + OTFA]^-$	$[M - OTFA]^-$
1	640	753	527
2	676	789	563
3, 4	700	813	587
5	668	781	555
6, 7	1038	1151	925
10	1006	1119	893
8, 9, 11	1024	1137	911
12, 13	992	1105	879

formed by loss of the aglycone fragment. This observed fragmentation was similar to that reported by König et al. [16] for EI-MS of trifluoroacetylated sugars. Other characteristic fragment ions of osidic moieties were observed and were summarized in Table 2. Therefore, sequence information was directly available from the spectra due to the successive cleavages, e.g. the molecular ion M^- (m/z 1038) and the fragment ions characteristic of TFA rutinose (m/z 901) and of TFA rhamnose (m/z 451) in the mass spectrum of linalyl rutinoside (Fig. 2). However, 6-O-β-D-apiofuranosyl and 6-O-α-L-arabinofuranosyl glucopyranosides could not be distinguished on the basis of the relative abundances of ions characteristic of the sugar moiety.

Structural information on the aglycone moiety was minimal except for molecular mass, which was calculated by difference between the molecular mass of glycosides and that of the sugar moiety which were both available. However, EI-MS of these TFA glycosides were shown to give valuable structural information on the aglycone moiety [9,10]. To our knowledge, overall results

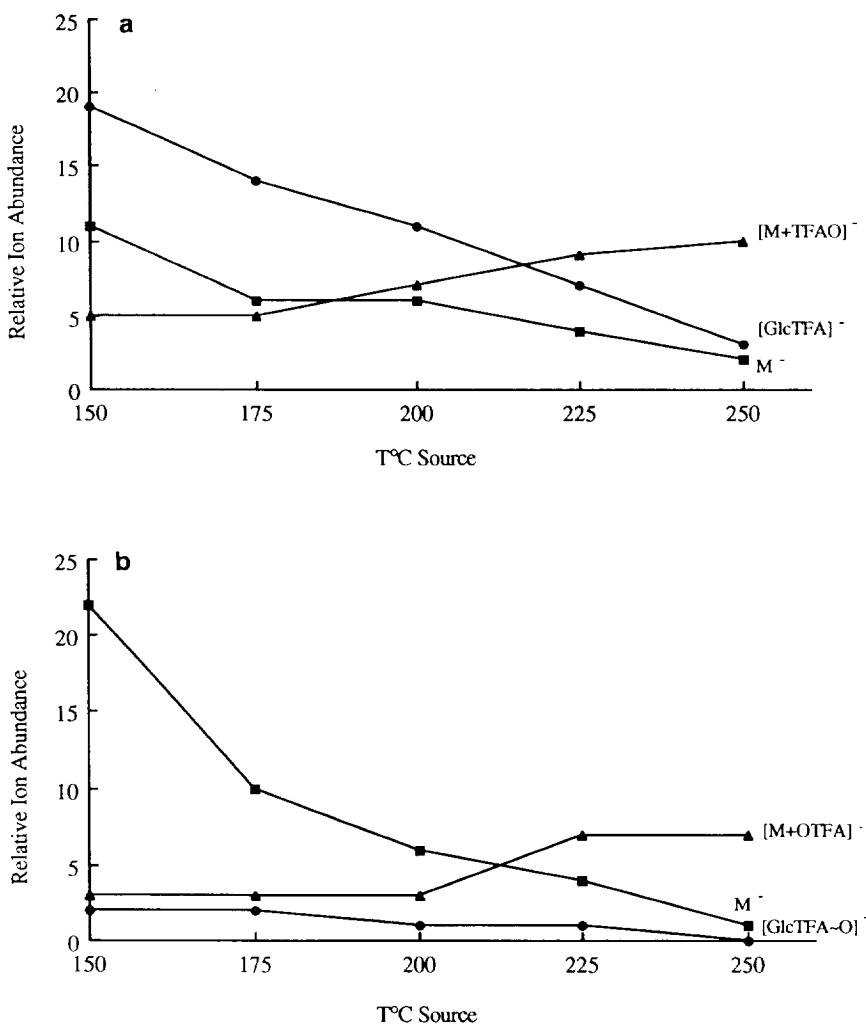

Fig. 3. Variation of ionic abundances of TFA derivatives of (a) phenyl β-D-glucoside and (b) octyl β-D-glucoside with source temperature.

Table 2
Interpretation of characteristic fragment ions of the sugar moiety

Glucoside		Apiosyl and arabinosyl glucosides		Rutinoside	
m/z	Fragment ions	*m/z*	Fragment ions	*m/z*	Fragment ions
563	$[GlcTFA \sim O]^-$	887	$[(Ara \sim Glc)TFA \sim O]^-$	901	$[RutTFA \sim O]$
677	$[GlcTFA \sim O + TFAOH]$	868	$[(Ara \sim Glc)TFA \sim O - F]$	882	$[RutTFA \sim O - F]$
544	$[GlcTFA \sim O - F]^-$	775	$[(Ara \sim Glc)TFA \sim O - (-H + TFAO)]$	789	$[RutTFA \sim O - (-H + TFAO)]$
451	$[GlcTFA \sim O - (-H + TFAO)]$	660	$[(Ara \sim Glc)TFA \sim O - [TFAO + TFAOH)]^-$	674	$[RutTFA \sim O - (TFAO + TFAOH)]^-$
		437	$[AraTFA \sim O]^-$	451	$[RhaTFA \sim O]^-$

afford valuable information for tentative identification of natural glycosides.

3.3. NCI-MS parameters effects

For a given moderating gas, the pattern of a CI spectrum can be strongly influenced by operational parameters such as ionization energy, source pressure and temperature [11,14,15]. Thus, the influence of these parameters on the relative abundances and the ionic current profiles of the M^- and $[M+TFAO]^-$ ions and of the $[GlcTFA]^-$ or $[GlcTFA\sim O]^-$ ions of TFA derivatives of phenyl and octyl glucosides were examined. The more significant parameter was the source temperature. When the source temperature increased, the relative abundances of the M^- ion and of the [GlcTFA] or [GlcTFA ~ O]$^-$ ions decreased, while the relative abundance of the adduct ion increased (Fig. 3). Furthermore, the molecular ion displayed the broadest chromatographic profile at the lowest source temperature (150°C) and at the highest pressure (186 Pa), which was ineffective to obtain a good reconstructed ion chromatogram. From these results, the operating conditions were chosen as follows: temperature and pressure of the source respectively at 200°C and 80 Pa; ionization energy at 200 eV.

3.4. Application to natural glycosides from passion fruit juice and muscat wine

Several papers have reported the occurrence of glycosides as flavor precursors in the juice of the purple passion fruit from analysis of the

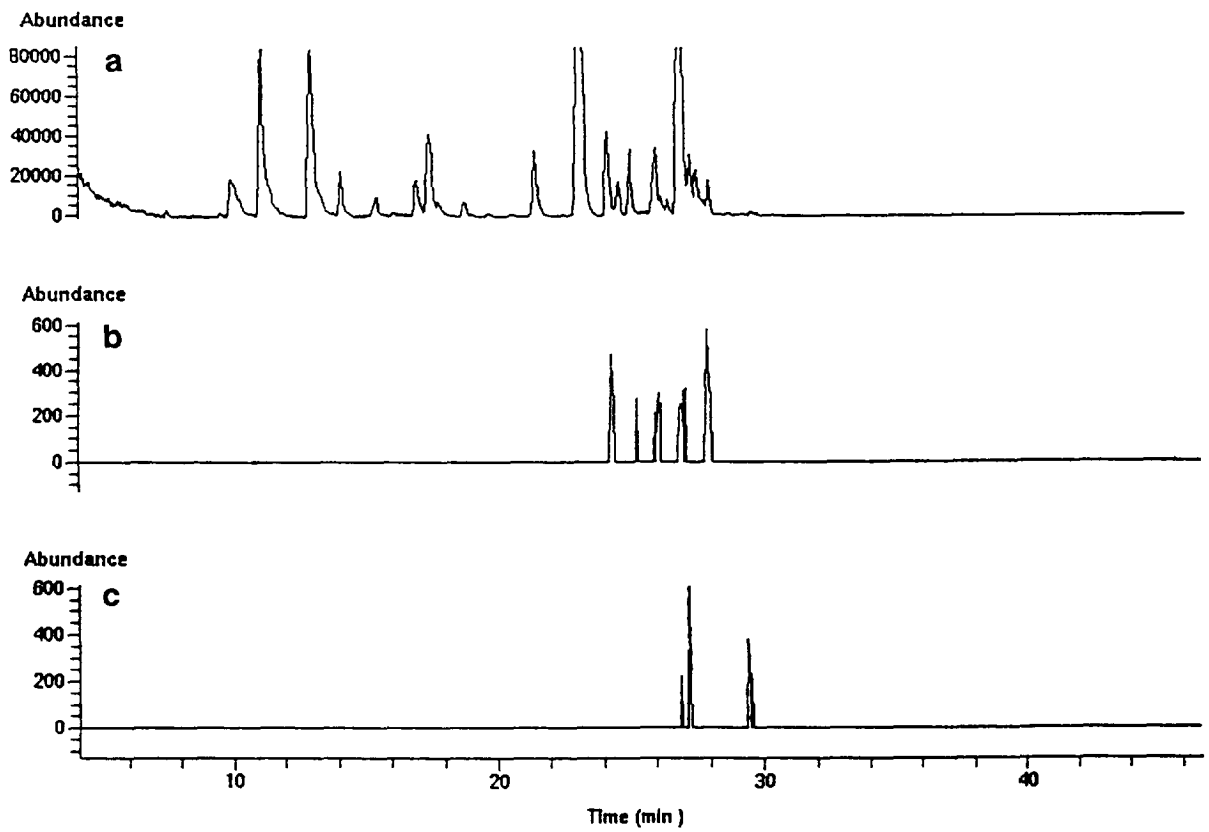

Fig. 4. GC–NCI-MS of a passion fruit glycosidic extract (TFA): (a) Total ion chromatogram, (b) reconstructed mass chromatogram at m/z 754 and (c) reconstructed mass chromatogram at m/z 770.

sugars and the aglycones liberated by acid or enzymatic hydrolysis of glycosidic extracts [4,5,17]. Some aglycones such as monoterpenoids with various oxidation states, benzyl alcohol, 2-phenyl ethanol and C-13 norisoprenoids were identified. However, there is some information about the nature of the sugars constitutive of the glycosidic moiety and their sequence is scarce. In contrast to passion fruit, glycosides of *Vitis vinifera* grape, as well as aglycones and sugar moieties, have been extensively investigated [1,2,6–10,18].

Thus, TFA derivatives of glycosides from passion fruit and muscat wine were analysed using GC–NCI-MS to obtain complementary informations to those reported by Voirin et al. [10] and Baumes et al. [18] from EI-MS of the TFA derivatives of grape and wine glycosidic extracts. The total ion current chromatograms (Figs. 4a and 5a) in the NCI mode of the TFA derivatives of the two fractions showed the same profiles as those obtained when the EI mode was used. Most of the glycosides detected in the chromatograms showed molecular ions at *m/z* 813, 1024 and 1038, which corresponded to glycosides of monoterpenols (M_r 154). Comparisons of spectra in the NCI and EI modes of the derivatives of the synthetic compounds allowed the positive identification of the corresponding natural glycosides. Furthermore, they allowed to obtain NCI-MS data for other natural glycosides. The cyanogenic glucoside, prunasin [2(*R*)-(β-D-

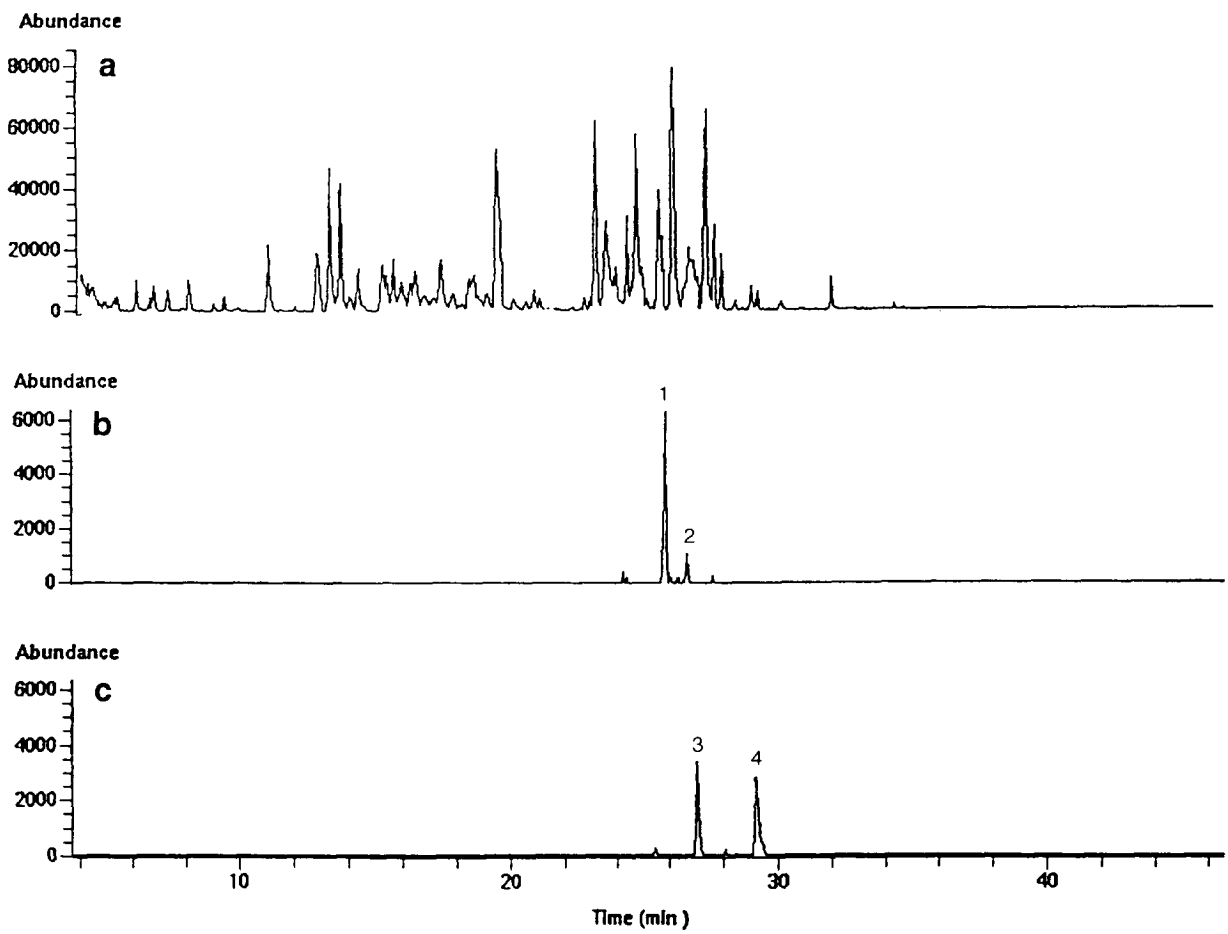

Fig. 5. GC–NCI-MS of a muscat wine glycosidic extract (TFA): (a) Total ion chromatogram, (b) reconstructed mass chromatogram at *m/z* 754 and (c) reconstructed mass chromatogram at *m/z* 770.

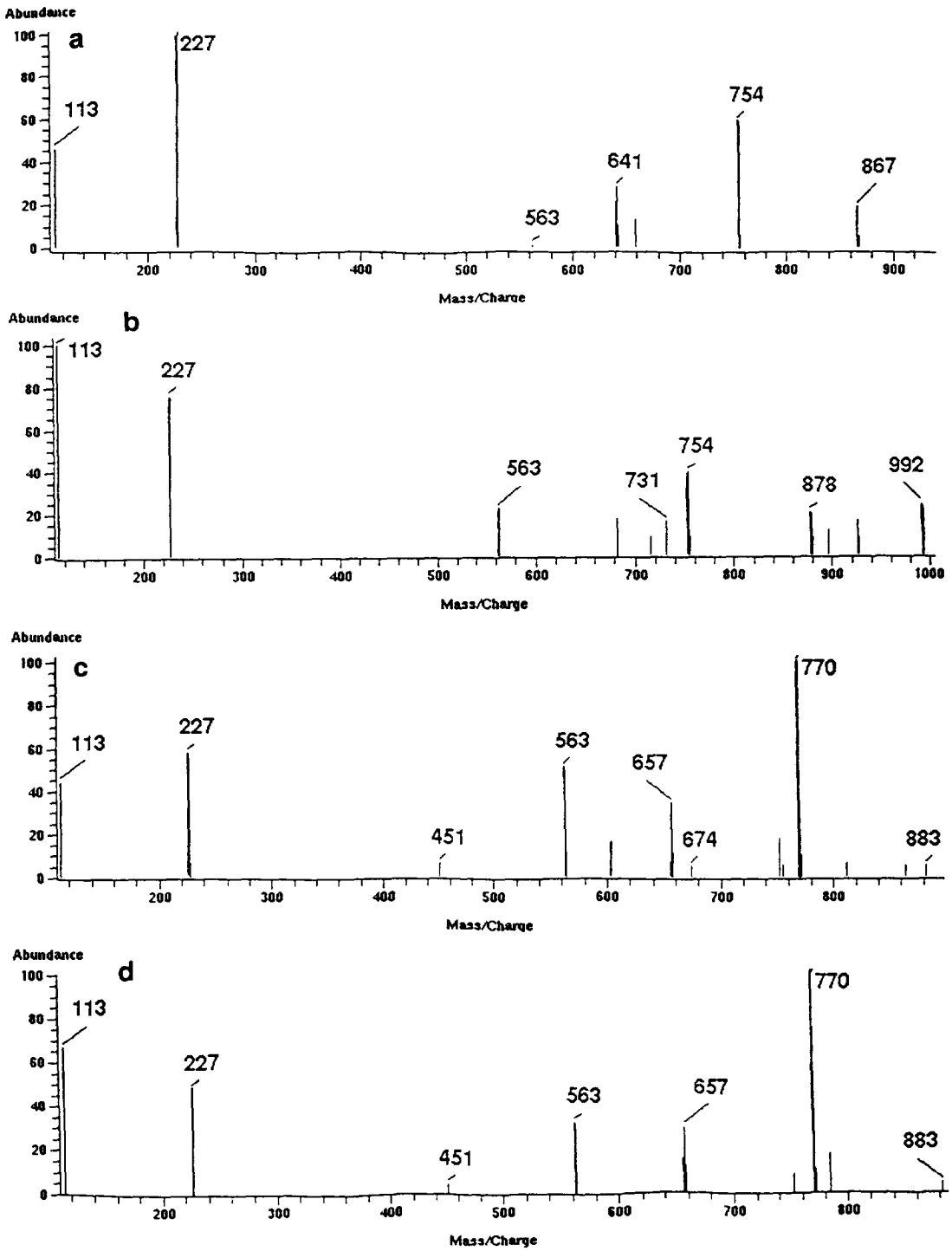

Fig. 6. NCI-MS spectra of TFA derivatives of four different C-13 norisoprenoïds of glucosides detected in muscat wine extract (a), (b) *m/z* 754 (peaks 1 and 2, Fig. 5b) and (c, d) *m/z* 770 (peaks 3 and 4, Fig. 5c).

glucopyranosyloxy)-2-phenylacetonitrile], identified in the purple passion fruit by Spencer et Seigler [19], gave some characteristic ions: M^- (m/z 679), $[M+TFAO]^-$ (m/z 792) and $[GlcTFA\sim O]^-$ (m/z 563). β-D-Glucopyranosides, β-D-rutinosides, α-L-arabinofuranosyl β-D-glucopyranosides of linalool oxides from the muscat wine extracts gave molecular ions at m/z 716, 1054 and 1040, respectively.

The detection of coeluted compounds was easier in NCI mode than in EI mode. As a lot of C-13 norisoprenoid aglycones of grape and passion fruit glycosides have been recently identified [18,20–22], we focused our attention on minor compounds as C-13 norisoprenoid glycosides. These compounds were difficult to detect because they were eluted in the range of the chromatogram corresponding to the most abundant monoterpene diglycosides. The peaks of the reconstructed mass chromatograms at m/z 754 (Figs. 4b and 5b) and at m/z 770 (Figs. 4c and 5c) for the passion fruit extract (Fig. 4) and the wine extract (Fig. 5) were highly indicative of C-13 norisoprenoid glucosides with aglycone molecular masses of 208 and 224, respectively. Indeed the ions m/z 754 and 770 correspond to the molecular ions M^- of the TFA derivatives of C-13 norisoprenoid glucosides at the oxidation level of hydroxy megastigmadienone and oxygenated hydroxy megastigmadienone, respectively. For the muscat wine extract, the spectra obtained for the two derivatives selected from the ion m/z 754 were very similar (Fig. 6a and b), as well as those for the two derivatives selected from the ion m/z 770 (Fig. 6c and d). These spectra exhibited characteristic fragmentation of TFA derivatives: ions at m/z 563 and 451 allowing to identify the osidic part, in this case glucose. Other adduct and fragment ions were also significantly present. In fact, the derivatized glucosides detected at m/z 754 were assigned to glucosides of 3-hydroxy-β-damascone (Fig. 6a) and 3-oxo-α-ionol (Fig. 6b), already identified by Baumes et al. [18]. However, the second one was coeluted with a phenylethyl diglycoside giving the ions m/z 992 (M^-), 879 ($[M-OTFA]^-$) and 683 ($[M-OTFA-HOTFA]^-$) in the spectrum shown in Fig. 6b.

For the passion fruit extract, four peaks were detected in the reconstructed mass chromatogram at m/z 754 and two peaks at m/z 770, but their abundances were lower than that obtained for the muscat wine extract. All these trace compounds, except the last eluted one, were coeluted with glycosidic compounds much more abundant (see the difference in the abundances of the peaks shown in Fig. 4a and Fig. 4b or c) so that the spectra obtained were very polluted. However the two peaks detected at m/z 770, as well as the second and the third eluted ones detected at m/z 754, had the same retention times as those detected in the wine extract. Thus, it may be assumed that these compounds are identical, which could be checked by detecting ion current at a few characteristic mass values by GC–EI-MS with selected ion monitoring of their TFA derivatives. These last results will be published in a forthcoming paper on glycosidic precursors of passion fruit.

4. Conclusions

We have shown that NCI mass spectra of the TFA derivatives of synthetic and natural glycosides of volatile compounds provided complementary useful informations for their structural determination. In contrast to EI-MS, NCI-MS allowed to obtain the molecular mass and the carbohydrate sequence of these glycosides. A good optimization of the source temperature was crucial for the direct observation of molecular ions. Thus GC–EI- and–NCI-MS are very suitable for on-line analysis of naturally occurring mono- and diglycosides of volatile compounds and could be used as screening techniques for these compounds.

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