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MASS SPECTROMETRIC STUDIES OF UNDERIVATIZED POLYPHENOLIC GLYCOSIDES

BRUNO DOMON and KURT HOSTETTMANN

Institut de Pharmacognosie et Phytochimie, Ecole de Pharmacie de l'Université de Lausanne, 2 rue Vuillermet, CH-1005 Lausanne, Switzerland

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Key Word Index—Flavonoids; xanthone glycosides; mass spectrometry; fast atom bombardment; desorption chemical ionization; field desorption.

Abstract—Flavonoid and xanthone glycosides were studied by mass spectrometry using soft ionization techniques such as desorption chemical ionization, fast atom bombardment and field desorption. In all cases a preliminary derivatization was not required. Information on the M_r, the sugar sequence and structure of the aglycone could be obtained.

INTRODUCTION

Mass spectrometric studies of polyphenolic glycosides using conventional ionization techniques (electron impact or chemical ionization) require a preliminary derivatization to increase the volatility and the stability of these compounds. Permethylation, peracetylation, pertrifluoroacetylation and pertrimethysilylation are used; all these methods have the disadvantage of increasing the M_r. Taking into account the large number of hydroxyl and phenolic groups in flavonoid glycosides, permethylation (or perdeuteromethylation), which gives the smallest increase in mass, is preferred [1]. These methods often produce mixtures of partially derivatized compounds which require subsequent purification. In some cases permethylation can produce artefacts, e.g. when ester groups are present. Usually only weak molecular ion signals are observed when permethylated compounds are studied under electron impact (EI) conditions [1]. However, intensities can be increased with chemical ionization using isobutane or ammonia as reactant gas [1]. Field desorption (FD) [2] was the first ionization technique employed for the study of polar and/or thermolabile compounds without derivatization; its application to flavonoids was reported by Schulten and Games [3]. Owing to the relative technological complexity of field desorption, other soft ionization methods have been developed in the last few years. Among them is desorption chemical ionization (DCI) [4, 5], which uses a probe consisting of an electrically-heated tungsten wire introduced into the chemical ionization source. Usually, ammonia is used as reactant gas. Secondary ion mass spectrometry (SIMS) [6] is an ionization technique using high energy (5–10 keV) argon or xenon ions, whereas fast atom bombardment (FAB) [7] works with neutral atoms. In the latter method, the sample is solubilized in a polar matrix, such as glycerol, and deposited on a copper target which is bombarded with energized atoms, inducing the desorption and the ionization. Plasma desorption (PD) [8] and laser desorption (LD) [9] are other soft ionization techniques which require mention.

DCI and FAB are methods of interest because they are commercial options available for most mass spectrometers.

In the present work, six underivatized flavonoid glycosides and one xanthone glycoside were studied by desorption chemical ionization, fast atom bombardment and field desorption. The results obtained from the different methods are compared. These spectra afforded information on the M_r, the sugar sequence and, in some cases, pertinent fragments of the aglycone.

RESULTS

The flavonoid glycoside robinin (**1**) was studied by DCI, FAB and FD; the results for the significant signals are summarized in Table 1. Conventional DCI, where the solid sample is deposited on the tungsten wire, gave poor results for the high mass ions in both positive and negative ion modes. Signals at m/z 741, $[M+H]^+$ and m/z 595 $[(M+H)-146]^+$, which correspond to the quasi-molecular ion and the fragment resulting from the loss of a rhamnosyl moiety, were not observed. Peaks at m/z 433 $[(M+H)-308]^+$ and at m/z 287 $[(M+H)-454]^+$ resulted from the loss of two and three sugar units, respectively. The corresponding fragments in the negative ion mode were observed at m/z 740 $[M]^{-\cdot}$ at m/z 595 $[M-146]^{-\cdot}$ m/z 432 $[M-308]^{-\cdot}$ and m/z 286 $[M-454]^-$. However, when the sample was solubilized in glycerol and deposited in solution on the probe, as previously described [10], the relative intensities of the ions of higher masses were considerably increased, as shown in Fig. 1. It should be noted that negative ion spectra generally gave more information than those in the positive ion mode.

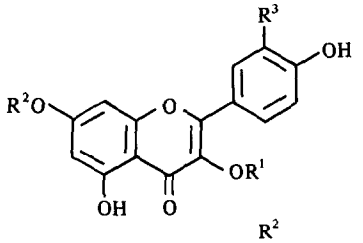
The fast atom bombardment mass spectra, in positive and negative ion modes gave similar results. Here too, the quasi-molecular ion and the higher mass fragments were more intense in the negative ion mode. The negative quasi-molecular ion $[M-H]^-$ at m/z 739 and fragments resulting from the splitting of one, two and three sugar units, were observed at m/z 593, 431 and 285, respectively.

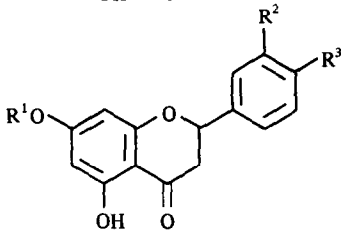
The FD mass spectrum gave the following information: the cationized quasi-molecular ions were observed at m/z

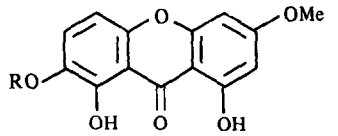
Table 1. Comparison of the DCI, FAB and FD mass spectra of robinin (1)

Ions	DCI using glycerol as matrix		FAB		FD	
	PI	NI	PI	NI		
Quasi-molecular [$\bar{M}$]	$[M+H]^+$	$[M]^-$	$[M+H]^+$	$[M-H]^-$	$[M+Na]^+$	$[M+K]^+$
m/z (rel. int., %)	741 (0)	740 (13)	741 (11)	739 (100)	763 (100)	779 (49)
[$\bar{M}-146$]	595 (20)	594 (100)	595 (9)	593 (98)	617 (59)	—
[$\bar{M}-308$]	433 (100)	432 (93)	433 (25)	431 (71)	455 (4)	—
[$\bar{M}-454$] (= aglycone)	287 (47)	286 (66)	287 (100)	285 (96)	286 (3)	—

PI, Positive ion mode; NI, negative ion mode.







1 R^1 = Rha(1–6) Gal R^2 = Rha R^3 = H
2 R^1 = Rutinosyl R^2 = H R^3 = OH
3 R^1 = Glc R^2 = Rha R^3 = OH
6 R^1 = Glur R^2 = H R^3 = OH

4 R^1 = Neohesperidosyl R^2 = H R^3 = OH
5 R^1 = Rutinosyl R^2 = OH R^3 = OMe

7 R = (4″- acetyl) Rha(1–6)Glc

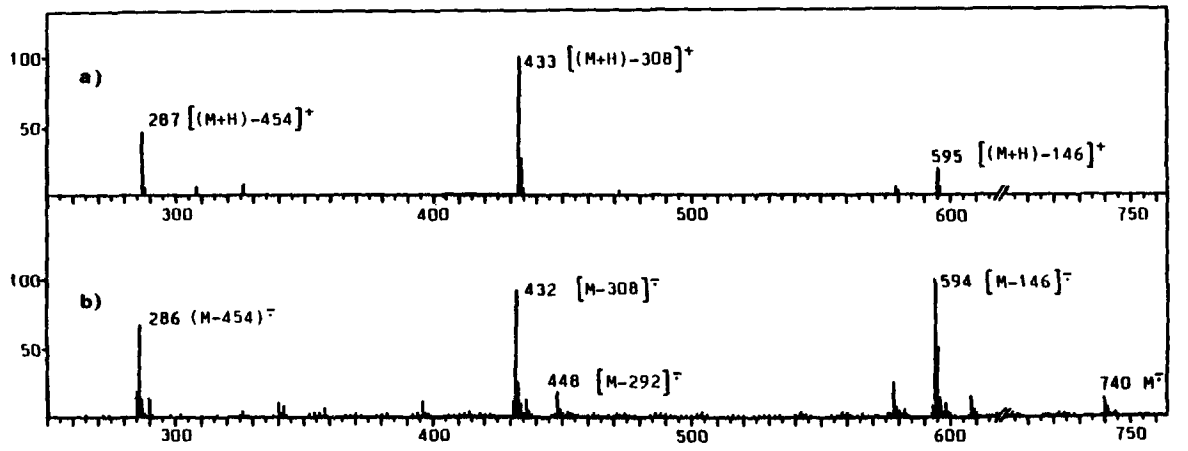


Fig. 1. DCI mass spectra of robinin (a) positive ion mode; (b) negative ion mode.

779 $[M+K]^+$ and m/z 763 $[M+Na]^+$ with strong intensities. The fragments after cleavage of the sugar units were present, but of lower intensity, as shown in Table 1.

Two isomers, rutin (**2**) and quercetin 3-*O*-α-L-rhamnoside-7-*O*-β-D-glucoside (**3**) were studied by DCI. From their positive ion spectra (see Fig. 2), it was possible to distinguish their different substitution patterns. Both compounds showed quasi-molecular ions $[M+H]^+$ at m/z 611. They both lost a rhamnosyl unit, corresponding to the signal at m/z 465 $[(M+H)-146]^+$. The elimination of a glucosyl moiety (which must be a terminal sugar) was only observed for quercetin 3-*O*-rhamnoside-7-*O*-glucoside. The loss of 162 amu was not observed for rutin because the glucosyl is an inner sugar moiety and can only be eliminated as a disaccharide or after preliminary splitting of the terminal sugar.

The aglycone signal was observed at m/z 303. The complementary fragments of the sugar moieties confirmed these structures; in the case where the two sugar units were attached independently to the aglycone, two signals were observed at m/z 164 $[\text{rhamnosyl}+NH_4]^+$ and m/z 180 $[\text{glucosyl}+NH_4]^+$, whereas for rutin, a signal was present at m/z 326 corresponding to the disaccharide–ammonia complex.

The flavanone glycosides hesperidin (**5**) and naringin (**4**) were studied by DCI, in positive and negative ion modes using ammonia as reactant gas. In all cases, the spectra showed the peaks of the quasi-molecular ion and those of the aglycone. For naringin (**4**) (Fig. 3a), signals in the positive ion spectrum for the quasi-molecular ions $[M+NH_4]^+$ and $[M+H]^+$ were present at m/z 598 and m/z 581, and at m/z 290 $[(M+NH_4)-308]^+$ and m/z 273 $[(M+H)-308]^+$ for the aglycone after loss of the rhamnosyl–glucosyl moiety. Fragments of the sugars were

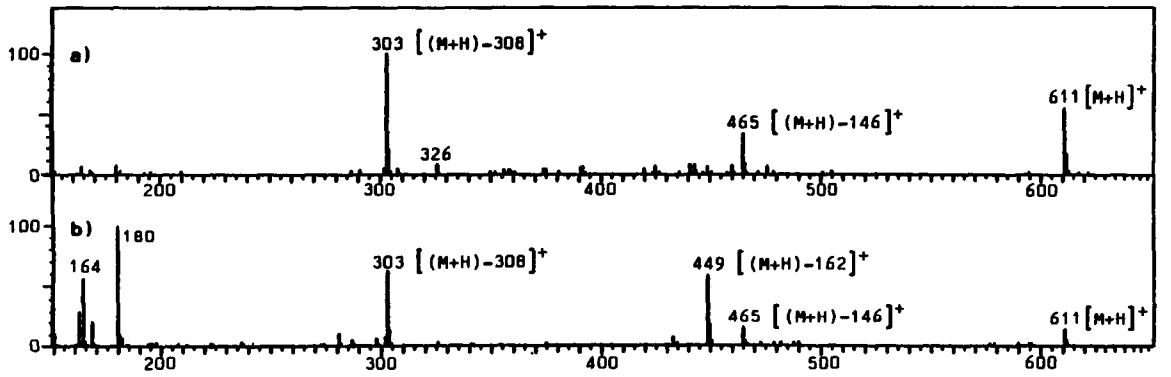


Fig. 2. DCI mass spectra of (a) rutin (**2**); (b) quercetin 3-rhamnoside-7-glucoside (**3**).

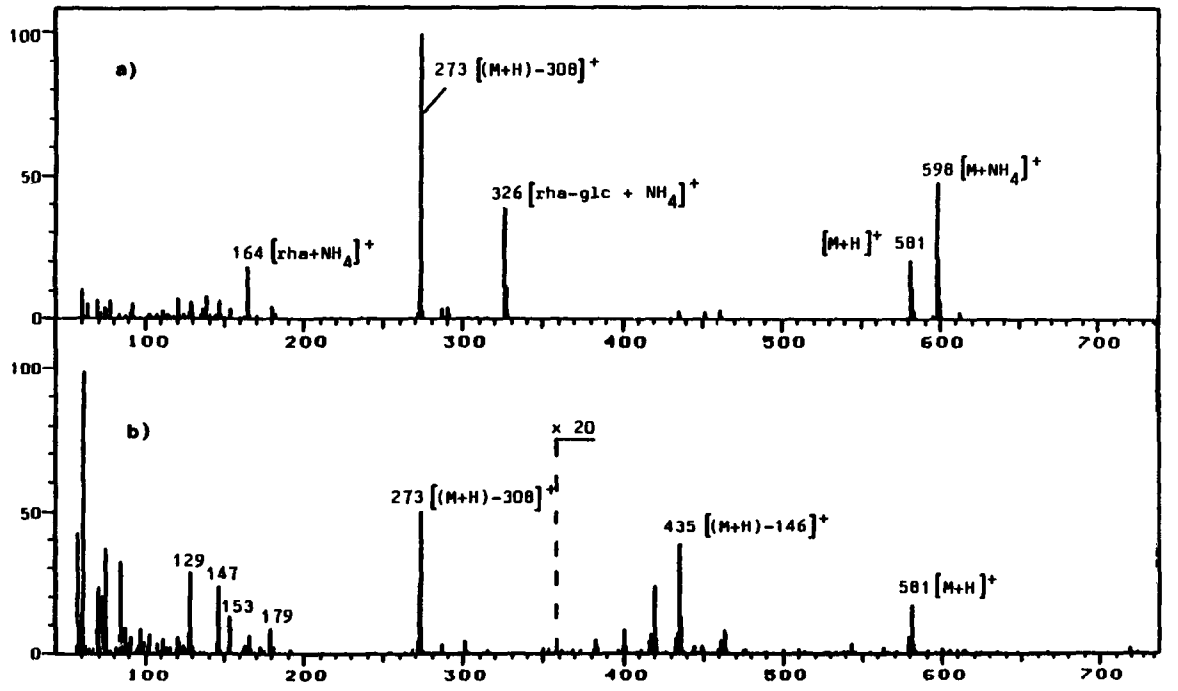


Fig. 3. DCI mass spectra of naringin (**4**): (a) reactant gas: ammonia; (b) reactant gas: methane.

observed at m/z 326 [rhamnosyl–glucosyl + NH_4]$^+$, at m/z 164 [rhamnosyl + NH_4]$^+$ and m/z 180 [glucosyl + NH_4]$^+$. The negative ion spectrum showed two main peaks, one at m/z 580 corresponding to the molecular ion [M]$^-$ and at m/z 272 [M − 308]$^-$ associated with the aglycone. A small signal at m/z 434 resulted from the splitting of the terminal rhamnosyl unit.

Hesperidin (**5**) which has the same disaccharide but an extra *O*-methyl substituent on ring B, showed similar spectra with a shift of 30 amu. Quasi-molecular ions [M + H]$^+$ and [M + NH_4]$^+$ were observed at m/z 611 and m/z 628 and the aglycone ions at m/z 303 [(M + H) − 308]$^+$ and m/z 320 [(M + NH_4) − 308]$^+$. An additional small signal at m/z 482 was present [(M + NH_4) − 146]$^+$. Sugar fragments were observed at m/z 326 and 164. The negative ion spectrum showed intense signals at m/z 610 [M]$^{-\cdot}$ and m/z 302 [M − 308]$^-$, corresponding to the molecular ion and the aglycone, respectively.

Significant fragments, informative for the substitution pattern of the aglycone, are observed when methane is used as reactant gas. Fragmentation pathways of flavonoid aglycones have been previously described by Kingston and Fales [11] and are summarized in Fig. 4. Pathways a and c lead to the same charged fragments for both compounds because they have identical substitution patterns for ring A. Pathway b affords different ions derived from ring B. The difference of 30 amu corresponds to the *O*-methyl substituent.

As shown in Fig. 3b, naringin gave signals at m/z 179, 153 and 147. The fragment at m/z 147 could also be derived from the rhamnosyl moiety [Rha + H]$^+$ and subsequent elimination of water could give the signal at m/z 129. In addition, the methane spectrum showed a quasi-molecular ion [M + H]$^+$ at m/z 581 and a fragment of low intensity arising after splitting of the terminal rhamnosyl moiety at m/z 435 [(M + H) − 146]$^+$.

Similar results were observed for hesperidin; signals resulting from the fragmentation of the aglycone were observed at m/z 179, 177 and 153. Additional peaks for the rhamnosyl moiety were present at m/z 147 and 129. Quasi-molecular [M + H]$^+$ and fragment [(M + H) − 146]$^+$ ions were observed at m/z 611 and 465.

Glucuronides are very polar compounds, the mass spectra of which are difficult to obtain. The quercetin 3-*O*-glucuronide (**6**) isolated from *Polygonum viviparum* L. was studied by desorption chemical ionization and fast atom bombardment. No molecular ion was observed in the DCI (positive ion) spectrum; nevertheless, two important fragments were present at m/z 303, corresponding to the protonated aglycone, and at m/z 194 arising from the adduct of one glucuronyl moiety with an ammonium ion.

The positive and negative ion FAB spectra of this compound were measured (see Fig. 5). The first one showed signals at m/z 501 [M + Na]$^+$ and m/z 479 [M + H]$^+$. The aglycone ion was observed at m/z 303 [(M + H) − 176]$^+$. The negative ion spectrum gave signals at m/z 499 [(M + Na) − 2H]$^-$, m/z 477 [M − H]$^-$ and m/z 301 [(M − H) − 176]$^-$. For glucuronides which give mass spectra only with difficulty, FAB seems to be a method of choice for their study.

Results obtained for the acetylated xanthone gentiabavarutinoside (**7**) [12] are shown in Table 2. Here, techniques which do not require a previous derivatization are very useful since the permethylation would cleave the ester bond and, after the peracetylation, the original acetyl group would no longer be distinguished from the other acetyl groups. The positive ion DCI spectrum showed the following signals: m/z 642 and 625 for the quasi-molecular ions [M + NH_4]$^+$ and [M + H]$^+$, m/z 587 [(M + H) − 42]$^+$ and m/z 275 [(M + H) − 350]$^+$.

The positive FAB spectrum gave only poor results but the most information was obtainable from the negative ion spectrum: m/z 623 [M − H]$^-$, the fragment obtained after loss of the acetyl–rhamnosyl moiety was observed at m/z 435 and the aglycone at m/z 273. The field desorption spectrum showed two important signals: the pseudo-molecular ion [M + Na]$^+$ at m/z 647 and the deacetylated compound [(M + Na) − 42]$^+$ at m/z 605.

DISCUSSION

Spectra obtained in the negative ion mode are generally

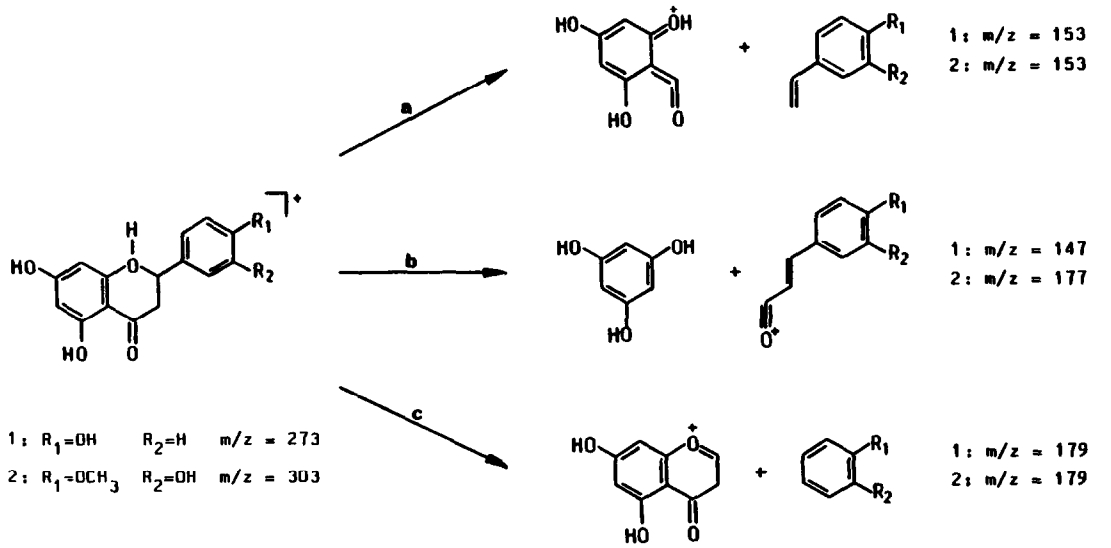


Fig. 4. Fragmentation pathways of flavanones.

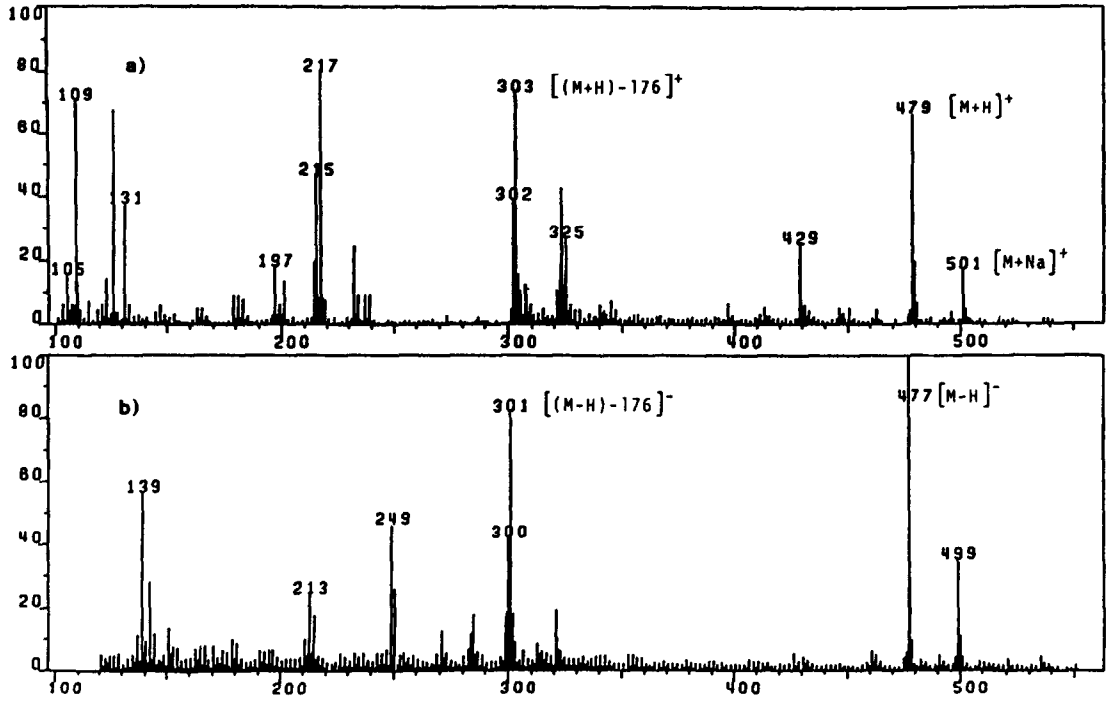


Fig. 5. FAB mass spectra of quercetin 3-*O*-glucuronide (6): (a) positive ion mode; (b) negative ion mode.

Table 2. DCI, FAB and FD mass spectra of gentiabavarutinoside (7)

	DCI	FAB		
Ions	PI	PI	NI	FD
Quasi-molecular [$\bar{M}$]	$[M+H]^+$	$[M+H]^+$	$[M-H]^-$	$[M+Na]^+$
m/z (rel. int., %)	625 (100)*	625 (6)	623 (100)	647 (100)
$[\bar{M}-42]$	583 (7)	—	—	605 (22)
$[\bar{M}-188]$	437 (0)	—	435 (37)	459 (2)
$[\bar{M}-350]$	275 (18)	275 (100)	273 (100)	297 (1)

PI, Positive ion mode; NI, negative ion mode.
* $[M+NH_4]$ *m/z* 642 (53%).

more useful; greater intensities result for high mass ions, especially for molecular or quasi-molecular ions. It should be noted that, in DCI, the molecular ion $[M]^{+\cdot}$ is obtained, whereas by the FAB technique the quasi-molecular ion $[M-H]^-$ is observed.

It is not easy to say which one of the three methods used in this work gives the best results as the methods are often complementary. Because of its relative simplicity, DCI is a very useful technique which quickly provides good positive and negative ion spectra. Nevertheless, for molecules having a MW over 900, obtaining a molecular ion can be a problem. FAB is an alternative method, useful for very polar and high mass compounds. The main disadvantage of this method is the production of interfering signals $[M_n+H]^+$ or $[M_n-H]^-$ from the matrix (M: glycerol or thioglycerol), which could complicate the interpretation of the spectra. In some cases, addition of acids or bases to the matrix can greatly increase the quality of the spectra [13].

The techniques of DCI- and FAB-mass spectrometry would appear to be good alternative methods for the study of polyphenolic glycosides without the need of preliminary derivatization. Similar results have been obtained for triterpene glycosides [Domon, B. and Hostettmann, K., unpublished results]. Information on the MW, the sugar sequence and the MW of the aglycone can be obtained from these spectra. In addition, fragments informative for the structure of the aglycone can be produced by desorption chemical ionization when methane is used as reactant gas. Isomers having different glycosidic substitution patterns can easily be distinguished by their mass spectra.

As shown in this work, mass spectrometry using soft ionization techniques is a very helpful tool in the structure

elucidation of flavonoid glycosides, especially as much information can be obtained with only a few micrograms of sample.

EXPERIMENTAL

DCI MS were measured on a Ribermag R-10-10B quadrupole spectrometer equipped with a Sidar data system (for details see Ref. [5]). FAB MS were obtained on a ZAB-1S spectrometer. The samples were suspended in glycerol or thioglycerol and the target was bombarded with 5 keV Xe atoms. FD MS were produced on a Varian MAT 731 apparatus equipped with a SS-200 data system. Carbon microneedle emitters were used; heating current was 20–40 mA.

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