28. Desorption/Chemical Ionization Mass Spectrometry of Naturally Occurring Glycosides[1])

by **Kurt Hostettmann**

Pharmazeutisches Institut der Eidgenössischen Technischen Hochschule, ETH-Zentrum, CH-8092 Zürich

and **Jacques Doumas** and **Michel Hardy**

Laboratoire d'Applications, NERMAG SA, 49, quai du Halage, F-92500 Rueil-Malmaison

(5.XI.80)

Summary

Desorption/chemical ionization mass spectrometry (D/CI.-MS.) is a recently developed technique especially indicated for highly polar and non volatile compounds. Various naturally occurring glycosides such as saponins, iridoid and secoiridoid glycosides, cardenolides and flavone-*O*-glycosides have been investigated by this method. All the measurements were carried out on underivatized compounds. In addition to the structural informations generally furnished by field-desorption mass spectrometry (molecular ion and sugar sequence), the molecular ion and pertinent fragments of the aglycone could also be obtained.

Introduction. – One of the major concerns for the chemist since the introduction of mass spectrometry as analytical technique consists in obtaining mass spectra of non volatile and/or thermally labile compounds. These compounds are of prime importance in natural products chemistry, biochemistry and medecine and thus, several methods have been elaborated.

Electron impact mass spectrometry (EI.-MS.) carried out on various derivatives has been widely used and numerous systematic studies have been reported. The aim of chemical derivatization is to transform the sample to be analyzed in a more volatile, less polar and thermally more stable compound. However, there is no universal procedure and derivatization is limited by the difficulty to obtain a single component from a polyfunctional molecule (permethylation) and the great increase of molecular weight (peracetylation). Trimethylsilyl derivatives of plant glycosides have been extensively investigated by gas-liquid chromatography combined with mass spectrometry [1].

The derivatization step is avoided in field desorption mass spectrometry (FD.-MS.) which is now a well-established technique for the analysis of glycosides

[1]) Presented in part at the 12th IUPAC International Symposium on the Chemistry of Natural Products, Sept. 21–27, 1980, Tenerife, Spain.

0018-019X/81/1/0297-07$01.00/0 © 1981 Schweizerische Chemische Gesellschaft

and other solid samples with little volatility [2–7]. FD.-MS. was introduced in 1969 by *Beckey* [8] and requires the use of emitters with an activated surface and extremely high electric fields for the desorption and ionization of the molecules of the sample in relatively soft conditions. The obtained spectra present generally the molecular ion, but little fragments. Thus, FD.-MS. provides relatively few structural informations. Excellent results in high molecular weight determination have been obtained by plasma desorption mass spectrometry (PD.-MS.) which involves ultrarapid heating of molecules deposited on thin nickel foil following impact by ^{252}Cf fission fragments [9]. But this technique is not of common use owing to the high complexity of the instrumentation.

The D/CI. method which has allowed to obtain the results presented below has been developed once it was found that the electric field in FD. was not downright necessary [10] [11].

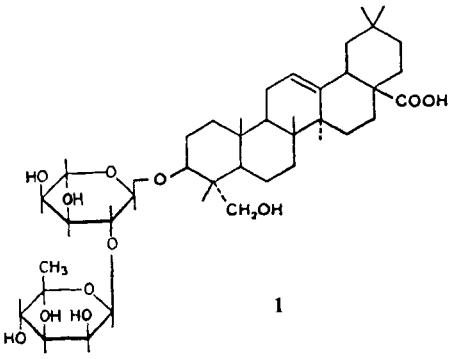
Recently, *Arpino & Devant* [12] and *Beaugrand* [13] presented D/CI.-MS. together with other soft ionization methods and reported some typical applications. As no naturally occurring glycosides have been investigated until now by this method, we submitted several selected underivatized glycosides belonging to different classes of substances to D/CI.-MS. in order to explore its suitability for structure elucidation of polar natural products. In general, much more structural informations than with FD.-MS. were obtained.

Method. – All the measurements were carried out on a quadrupole MS. equipment and a CI. source connected to an ammonia feedline. The emitter was a coiled tungsten wire where the solubilized sample was applied (1 µl of a 1 µg per µl solution). The coiled wire is fixed on the extremities of a special probe inlet system, introduced in the ionization source and heated by a programmable current until desorption occurs (adjustable from 1 mA to 1 A which corresponds to room temperature up to 1200°, at a speed of 1 to 99 mA/s). The analysis consists to heat rapidly the emitter and to record continuously mass spectra with the help of the data system associated to the MS. instrument. The temperature of the walls of the source is kept as low as possible. Visualization of the total ionic current allows to follow the whole desorption process.

The obtained spectra are CI. spectra which present always the molecular or pseudo-molecular peak as well as characteristic fragments of the molecule. Ammonia is generally used as reactant gas since its provides a softer ionization than methane or isobutane and thus, gives the highest probability to get molecular weight informations. The molecular ion and main fragments are often associated with NH_4^+. The informations furnished by spectra recorded during the course of the desorption are noticeably different from spectra to spectra. The relative abundance of the molecular ion is more important in a spectrum recorded at the beginning of the desorption than at the end; on the other hand, spectra are more 'fragmented' near the end of the desorption. This is due to the constant increase of the emitter temperature which modifies considerably the desorption and ionization conditions between the beginning and the end of the desorption. Furthermore, it is likely probable that pyrolysis phenomena appear when the temperature is too high at the end of the operation.

Results. – Several glycosides, previously isolated in our laboratory have been submitted to D/CI.-MS. analysis.

Saponins. Various triterpenoid and spirostanol glycosides have recently been studied by FD.-MS. [2] [3] [14] [15]. In order to compare FD.-MS. with the new technique of D/CI.-MS., we investigated several typical saponins, namely the molluscicidal principles of *Hedera helix* L. [14] and *Cornus florida* L. [15]. The D/CI.-MS. spectra of hederagenin 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (**1**) is shown in *Figure 1*. The top spectrum (mass range 470–830) has been recorded shortly after beginning of the desorption. The base peak at *m/z* 768 corresponds to $(M+NH_4)^+$. An intense $(M+H)^+$ peak is present at *m/z* 751, whereas the fragment with *m/z* 622 is due to $[(M+NH_4)-146]^+$ and thus, indicates that rhamnose is the terminal sugar. The fragments with *m/z* 472 and 490 correspond to hederagenin and hederagenin associated with NH_4^+. No further fragments were present.



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Mol-Wt.	750
Hederagenin	472
Arabinosyl	132
Rhamnosyl	146

The bottom spectrum (mass range 110–830) was recorded near the end of the desorption. It is noteworthy that in the mass range 470–830, we observe the same sequence than in the spectrum recorded at an earlier stage of the desorption process, but all fragments show smaller intensities. The base peak at *m/z* 164 corresponds to a rhamnosyl unit (terminal sugar) associated with NH_4^+. The fragment at *m/z* 296 corresponds to the disaccharide moiety with the NH_4^+ additional ion. All important aglycone fragments (*m/z* 455, 437, 248, 204, 189, 180) are also present and are characteristic of an oleanene type triterpene with a C(12), C(13) double bond [16]. The structural informations furnished by D/CI.-MS. include the molecular weight and the sugar sequence determination as well as important facts for the aglycone identification, whereas by FD.-MS. only the molecular ion was obtained for the same saponin [3]. A closely related saponin, hederagenin 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside (**2**), submitted to D/CI.-MS., afforded similar structural informations. In the spectrum recorded near the end of the desorption, the base peak was *m/z* 180 and corresponds to the terminal sugar moiety. This observation could be of diagnostic interest for the sugar sequence determination. The spectra of other *Hedera* saponins and of sarsapogenin glycosides [15] were also recorded and afforded the structural informations mentioned above.

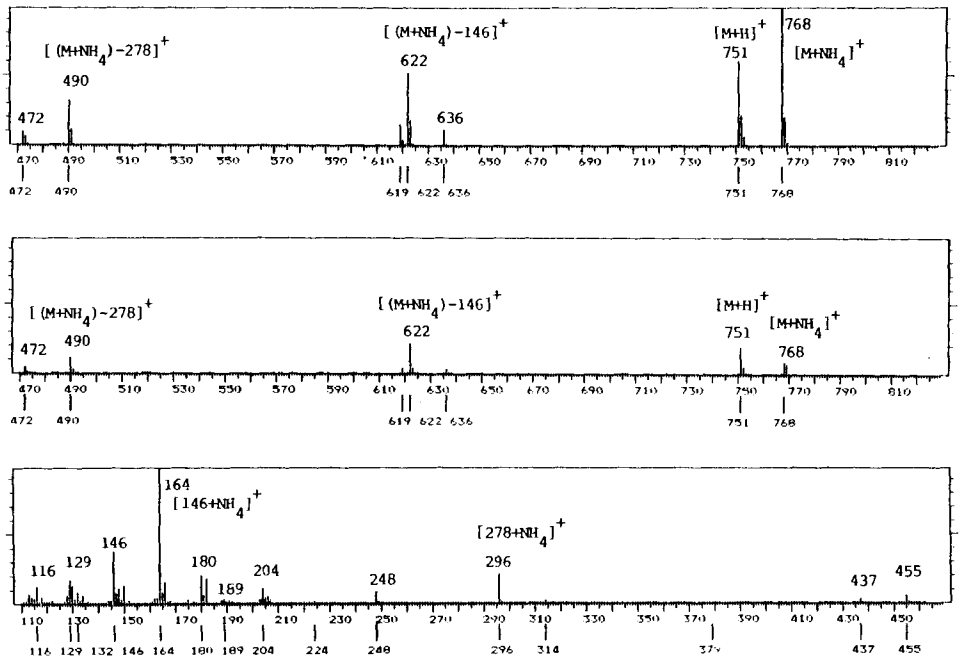


Fig. 1. *D/CI.-MS. analysis of hederagenin 3-O-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside* (**1**). The top spectrum (mass range 470–830) was recorded at the beginning of the desorption. The bottom spectrum (mass range 110–830) was recorded near the end of the desorption.

Iridoid and secoiridoid glycosides. Mass spectrometry of monoterpene glycosides and related compounds is usually carried out on peracetylated, permethylated or silylated derivatives. A systematic GC.-MS. study of trimethylsilyl derivatives has been reported by *Inouye et al.* [17]. Underivatized glycosides have also been studied as the aglycones were formed in the mass spectrometer by cleavage and H-transfer, but no molecular ion was obtained [18]. FD.-MS. affords the molecular ion, but generally no further fragments [19]. Typical iridoid glucosides have been submitted to D/CI.-MS. The results are summarized in the *Table.* The base peak consists always in the molecular ion associated with NH_4^+. The aglycone peaks were present in all the studied examples. In addition to the data indicated in the *Table*, each spectrum exhibited some specific aglycone fragments as well as peaks with *m/z* 162 and 180 due to the glucosyl moiety.

Table. *D/CI.-MS. data of some iridoid glucosides* (the relative abundance is given in parentheses)

	$(M+NH_4)^+$	M^+	$[(M+NH_4)-162]^+$	$(M-162)^+$
Aucubin	364 (100)	346 (3)	202 (6)	184 (14)
Catalpol	380 (100)	362 (6)	218 (7)	200 (6)
Harpagide	382 (100)	364 (2)	220 (15)	202 (6)
Loganin	408 (100)	390 (1)	246 (25)	228 (12)

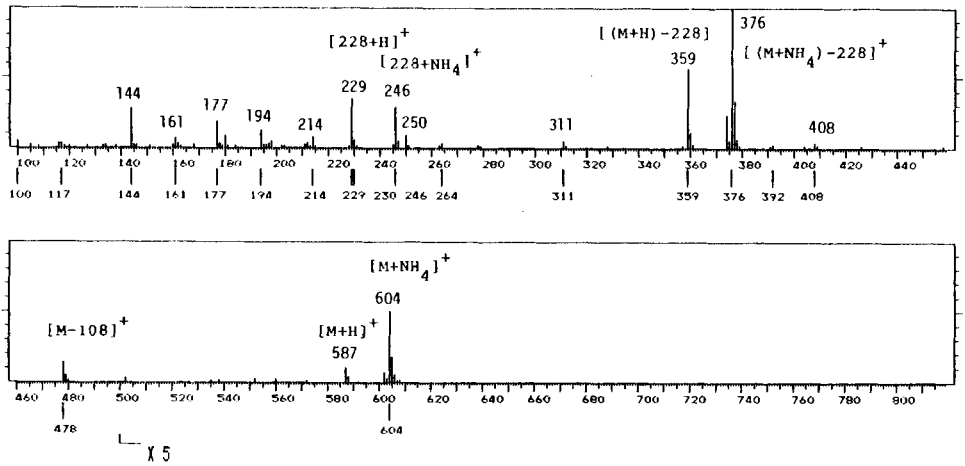
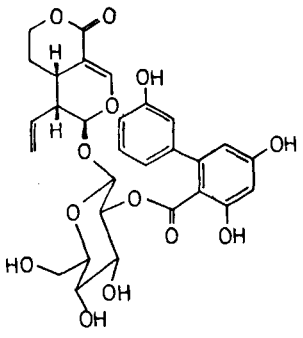


Fig. 2. *D/CI.-MS. spectrum of the secoiridoid glucoside amarogentin* (3) (mass range (100–820))

As both, molecular ion and aglycone peaks are obtained, D/CI.-MS. becomes a method of prime importance in the structure determination of iridoid and secoiridoid glycoside esters. This is illustrated in the following by the D/CI.-MS. analysis of amarogentin (**3**), one of the bitter principles of *Gentianaceae (Fig. 2).* The fragments with *m/z* 604 and 587 due to $(M+NH_4)^+$ and $(M+H)^+$, respectively, confirm the molecular weight. The base peak at *m/z* 376 corresponding to $[(M+NH_4)-228]^+$ and the fragment *m/z* 359 can be explained by the loss of the biphenyl moiety and thus, establish the sequence secoiridoid-glucosyl biphenyl-carboxylate. Pertinent fragments of the aglycone and the biphenyl moiety are also present [20].

In order to explain each fragment of the spectrum mentioned above, a D/CI.-MS. study of closely related secoiridoid glycosides is currently in progress.

Cardenolides. These glycosides being related to saponins, similar structural informations can be obtained. For example, the well-known cardiac glycoside digitoxin (**4**) (Mol-Wt. 764) formed of digitoxigenin and 3 digitoxosyl units has been submitted to D/CI.-MS. The spectrum shows mainly four couples of strong



Amarogentin (**3**)

Mol-Wt.	586
Aglycone	195
Glucosyl	162
Diphenyl moiety	229

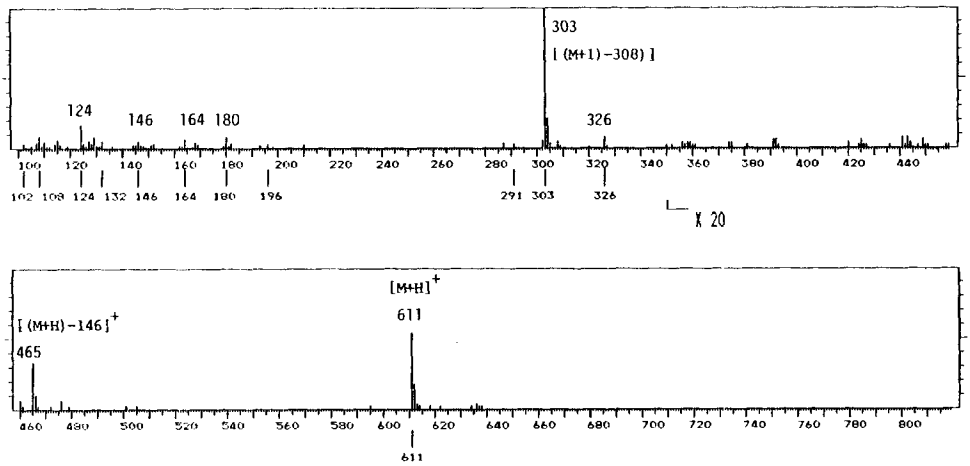


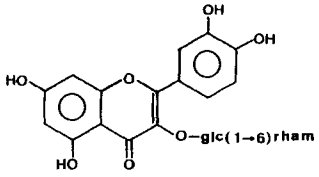
Fig. 3. *D/CI.-MS. spectrum of the flavonoid-*O*-glycoside rutin* (**5**) (mass range 100–820))

peaks at *m/z* 782 and 765, 652 and 635, 522 and 505, 375 and 392, corresponding respectively to $(M+NH_4)^+$ and $(M+H)^+$, $[(M+NH_4)-130]^+$ and $[(M+H)-130]^+$, $[(M+NH_4)-260]^+$ and $[(M+H)-260]^+$, $[(M+NH_4)-390]^+$ and $[(M+H)-390]^+$. In addition, some aglycone fragments could also been observed. We are presently investigating cardenolides possessing up to five sugar units in order to establish their sequence.

*Flavone-*O*-glycosides.* A typical D/CI.-MS. spectrum of a representative of this very important class of naturally occurring glycosides, rutin (**5**), is shown in *Figure 3.* It is noteworthy that the molecular ion (*m/z* 611) is not associated with NH_4^+. The peak at *m/z* 465 is due to the loss of a rhamnosyl unit and confirms the sequence quercetin-glucose-rhamnose.

The base peak *m/z* 303 corresponds to the aglycone quercetin associated with H^+. In all the investigated flavonoids, the molecular ion is weak. In the present case, it has been amplified by 20. A systematic D/CI.-MS. study of various flavonoid glycosides is currently in progress and will be published separately.

Discussion. – D/CI.-MS. has proved to be a suitable method for the analysis of underivatized naturally occurring glycosides. The molecular weight and the sugar sequence could be determined. In addition, when spectra were recorded near the end of the desorption, useful informations for the aglycone identification were also obtained. The obtention of different spectra during the course of the desorption



Rutin (**5**)

Mol-Wt.	610
Quercetin	302
Glucosyl	162
Rhamnosyl	146

presents a great interest. Selection of a spectrum at beginning of the desorption can be used to ascertain the molecular weight (base peak due to molecular ion), whereas spectra recorded later in the course of the desorption present more fragments for the structure elucidation.

The technique of D/CI.-MS. is more simple that FD.-MS. as it does not require high intensity electric fields and special emitter preparation, and generally, furnishes more structural informations. However, with the instrumentation used in the present study, no high resolution MS. can be made.

A systematic D/CI.-MS. study of more complex glycosides is currently in progress in order to determine the possibilities and limits of the method.

Experimental Part

All spectra were recorded on a *Ribermag* R 10-10B quadrupole mass spectrometer associated with a Ribermag SIDAR data system. The samples were dissolved in methanol at a concentration of 1 µg/µl and deposited with a 10 µl syringe (1–2 µl) on the tungsten wire. After evaporation of the solvent at room temperature, the D/CI. probe was introduced through the standard vacuum lock of the apparatus into the source. The intensity of the current in the emitter is programmed from 30 to 500 mA, at a speed of 7 mA/s. Desorption generally occurred when the intensity reached 250–300 mA (corresponding to 150–200°). The source temperature was in the range 100–150°. The pressure of the reactant gas was maintained at a constant value between 0.1 and 0.5 Torr. All the spectra were recorded with an integration time of 1 ms per mass unit. Between two runs, the filament was heated up to 1200° in order to clean the emitter.

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