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Note

Determination of the sugar linkage position in glycosides using ammonia chemical ionization mass spectrometry at low gas pressure

Gottfried Reznicek *, Marcel Mathà

Institute of Pharmacognosy, University of Vienna, Center of Pharmacy, Althanstraße 14, A-1090, Vienna, Austria

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In a previous publication [1], we reported on the determination of the linkage positions of sugar moieties in naturally occurring glycosides by GLC–MS after hydrolysis of the permethylated glycoside and trimethylsilylation of the carbohydrate derivatives.

Using electron impact mass spectra (EIMS) a typical fragmentation pattern was obtained [2–5] allowing the identification of the substitution at C-1–C-4 and the determination of the ring size of each sugar component. Unfortunately, the fragment responsible for the determination of the substitution at C-6 for hexoses appears with low intensity, therefore the identification of any linkage at this position was not possible [5]. Furthermore, the EI-mass spectra are characterized by the absence of molecular ions, that are essential for the correct identification.

In contrast to this behavior, chemical ionization mass spectrometry (CIMS) with the reactant gas ammonia is a complementary technique giving attachment ions (MNH_4^+) as base peaks, and the molecular weight can easily be deduced [6–8]. However, CIMS alone is of limited value, giving too few characteristic fragment ions.

From this point of view, the simultaneous generation of EI and CI ionization conditions seems to be of interest. Such an apparatus had been introduced [9] containing two ion sources requiring a large technical expense.

* Corresponding author.

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Therefore, it was the aim of this work to create experimentally mass spectra containing the information from both electron impact as well as chemical ionization mass spectrometry in one step using a conventional GLC–MS apparatus.

1. Results and discussion

Starting with pure CI-conditions (Table 1, **A**) a continuous decrease of the reactant gas NH_3 results in a decrease of the quasimolecular ion (*m/z* 558, CI-information) and an increasing fragmentation (EI-information). As soon as the reactant gas is introduced into the ion source using a very low pressure (less than 0.05 bar), the resulting spectra (Table 1, **B**) resemble EI-mass spectra (Table 1, **C**) showing the typical EI-fragments (*m/z* 191, 204, 217) and no MNH_4^+ ion. Between these two extremes, a certain adjustment of the reactant gas pressure is possible, yielding mass spectra with the typical EI-fragments as well as the quasimolecular ion with rather equal intensities (Table 1, **D**).

Most of the CI-mass spectra of sugar derivatives show fragments of variable intensity depending on thermolysis, different reactant gases, gas pressures, and/or difference in electron energies [7,8,10–13]. We intentionally used these incomplete CIMS conditions as a method permitting the acquisition of mass spectra with EI- as well as CI-information.

The adjustment of the low reactant gas pressure has to be performed very accurately in order to obtain reproducible results, as slight variations cause severe changes in the relations of the ion intensities.

A quick and precise tuning is possible by using the reactant gas itself, taking into consideration the creation of the reactant ion [8]:

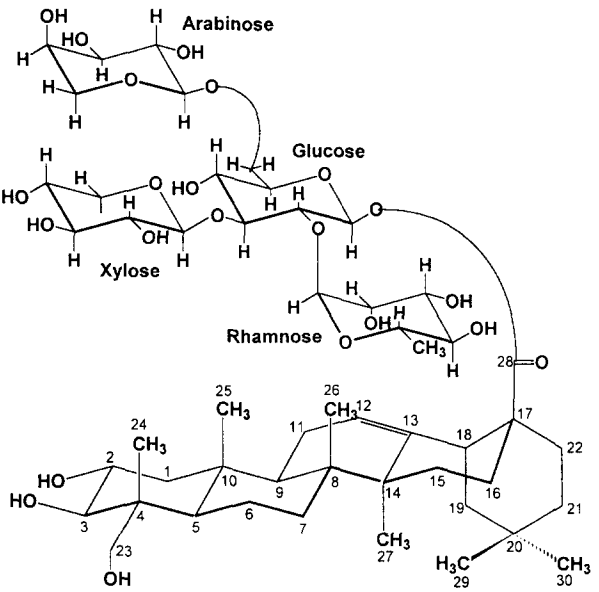
$$NH_3 + e^- \rightarrow NH_3^{+\cdot} + 2e^-$$

$$NH_3^{+\cdot} + NH_3 \rightarrow NH_2^{\cdot} + NH_4^+$$

Under CI-conditions, the excess of ammonia in the ion source reacts with all the initially formed $NH_3^{+\cdot}$ to give NH_4^+. Therefore, only a peak at *m/z* 18 can be observed. If the ammonia pressure is lowered, not all initially formed $NH_3^{+\cdot}$ ions are effectively intercepted and surviving $NH_3^{+\cdot}$ ions can be seen. Therefore, the abundance of the *m/z* 18 ion decreases corresponding to the increase of *m/z* 17 ($NH_3^{+\cdot}$).

Table 1
Main fragment intensities of ammonia CIMS with different reactant gas pressures, and of EIMS of pertrimethylsilylated glucose

	Mode	*m/z*					
		191	204	217	361	468	558
A	CI NH_3, 1 bar	–	–	–	5	18	100
B	CI NH_3, < 0.05 bar	78	100	29	28	–	–
C	EI	41	100	33	–	–	–
D	CI NH_3, ~ 0.1 bar	48	94	30	57	32	100



Scheme 1. Structure of heteropappussaponin 2.

Based on systematic investigations, the gas pressure should be adjusted to achieve a *m/z* 17:*m/z* 18 intensity ratio of 1:10 for recording optimal mass spectra.

The MS technique as presented above was used for the determination of the sugar linkage positions of heteropappussaponin 2 [14], a monodesmosidic triterpensaponin with an unusual sugar moiety (Scheme 1). After permethylation, hydrolysis, and trimethylsilylation according to ref. [1], the sugar derivatives were separated by GLC and analyzed by CIMS with ammonia at low pressure as mentioned above. The spectra of the terminal rhamnose, xylose, and arabinose showed the typical EI-fragments [1] (*m/z* 88, 101, 133) as well as the attachment ions MNH_4^+ (*m/z* 282 for arabinose and xylose, *m/z* 296 for rhamnose) in equivalent intensities. Furthermore, the resulting MS of the central glucose gave very important information (Fig. 1): The fragments *m/z* 191, 204, and 159 showed this sugar to be a pyranose with linkages at positions 1, 2, and 3. These facts also would have been evident using EI-mass spectra only; however, the determination of the substitution at C-6 for this sugar would not have been possible. The CI-mass spectrum, using ammonia at low pressure, additionally gave the molecular weight of this carbohydrate ($MNH_4^+ = m/z$ 500, therefore mol wt = 482) showing this compound to be a hexose with a linkage site at C-6. The other fragments are of low interest for the identification of this sugar derivative (*m/z* 410 is created by the loss of $Me_3Si–OH$ from MNH_4^+). Therefore a single GLC–MS analysis allowed the indubitable determination of the sugars and their linkage positions, which was of great importance for the elucidation of the sequence of the sugar moiety of heteropappussaponin 2 [14].

The reliability of this technique was proved by analysing the numerous glycosides and carbohydrates mentioned in literature [1]. All these analyses yielded mass spectra containing both EI- as well as CI-information similar to that shown above (Fig. 1). After

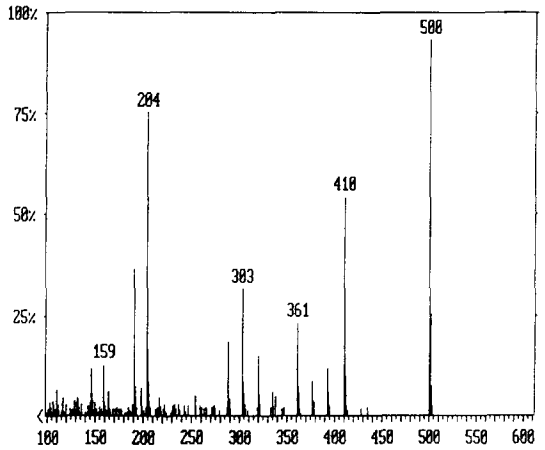


Fig. 1. CIMS of 4-*O*-methyl-1,2,3,6-tetra-*O*-(trimethylsilyl)-D-glucopyranose at low ammonia pressure.

repeated adjustment of the reactant gas pressure the intensities of the EI-fragments varied ±3.5%, the deviation of the quasimolecular ions was ±7.5% at the maximum.

As there is no apparatus available employing simultaneously electron impact and chemical ionization, the introduced method is a simple possibility applicable to conventional GLC–(CI)MS instruments. The use of this technique to identify the sugars and their linkage positions in glycosides demonstrates the suitability of the ammonia CI-technique at low reactant gas pressure for the analysis of complex natural products.

As ammonia is a convenient CI-reactant gas for compounds giving no molecular ions using EIMS, this method is certainly applicable to further substances with minor adaptations.

2. Experimental

Heteropappussaponin 2 {*O*-α-D-arabinopyranosyl-(1 → 6)-*O*-[α-L-rhamnopyranosyl-(1 → 2)]-*O*-[β-D-xylopyranosyl-(1 → 3)]-β-D-glucopyranosyl arjunolate} was isolated from *Heteropappus biennis* (LDB.)TAMAMSCH., mp 312–315 °C, $[\alpha]_D^{20}$ −3.3° (*c* 0.35, MeOH).

The analyses were carried out with a Shimadzu QP-1000 EX GLC–MS system, equipped with an EI/CI-ion source. The GLC separation was done with a fused silica capillary column SE 54-CB (Macherey Nagel), 50 m × 0.25 mm i.d., film 0.45 μm, with helium 5.0, flow 5 mL/min, as mobile phase. The split ratio was 1:5, open after 0.5 min, injector temperature 250 °C, interface 250 °C, and a temperature gradient 100–250 °C with rate 3 °C/min was used.

For EIMS the ion source temperature was 180 °C, 20 eV, vacuum 9×10^{-6} torr and scan 50–600 amu/2 s. For CIMS, NH_3 (2.8), pre-pressure 760 torr (1 bar), was used as reactant gas, the ion source temperature was 170 °C, 200 eV, vacuum 8×10^{-5} torr, and scan 80–700 amu/2 s.

To carry out CIMS at low reactant gas pressure, NH_3 (2.8), pre-pressure approx. 76 torr (0.1 bar), was employed with the ion source at 170 °C, 200 eV, vacuum approx. 3×10^{-5} torr, and scan 80–700 amu/2 s. To get reproducible CI-mass spectra at low pressure, the gas pressure must be adjusted to achieve a *m/z* 17 (NH_3^+):*m/z* 18 (NH_4^+) ion intensity ratio of 1:10.

The methylation, hydrolysis and trimethylsilylation of the glycosides was carried out according to ref. [1].

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