Exact Mass Measurements for α-Allenic Alcohol by Atmospheric Pressure Chemical Ionization/Time-of-flight Mass Spectrometry

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The atmospheric pressure chemical ionization/time-of-flight mass spectrometry (APCI/TOF-MS) was applied to determine the mass of five α-allenic alcohols via their protonated molecular ions using positive ion mode. Polyethylene glycol (PEG) was used as the internal reference. All results were obtained under the resolution of about 5000 FWHM (full width at the half maximum). Solvent effects were studied and the satisfied results were obtained in acetonitrile. Compared with the theoretical values, all absolute errors were less than 1.0 mmu. The effects of nozzle potential, push pulse potential, pull pulse potential, pull bias potential and acquisition rate on exact mass determination were also discussed. APCI/TOF-MS is proven to be a very sensitive analytical technique and an alternative ionization mode in analyzing thermally labile compounds with relatively weak polarity, such as α-allenic alcohol.

Keywords   exact mass measurement, atmospheric pressure chemical ionization/time-of-flight mass spectrometry, α-allenic alcohol

Fig. 1   Structures of the compounds analyzed.

Introduction

α-Allenic alcohols are an important kind of useful compounds in synthetic chemistry because of their unusual structures and have been widely applied to the synthesis of three- or five-membered oxygen-containing cyclic compounds.[1-4] The structures of five α-allenic alcohols are shown in Fig. 1.

Their molecular structures become unstable when ionized by electron impact (EI), thus the detection of their molecular ions M+ is always not easy. So it is difficult to determine their exact molecular mass by EI (Fig. 2). When they are ionized by chemical ionization (CI, methanol as the reagent gas), many adduct ion peaks appear, causing more difficulties in determining their exact masses of MH+ (Fig. 3). Because their protonated molecular ion peaks were weak and there were many adduct ions and background (m/z: 177, 217, 231 etc.) with high intensity, it was difficult to select the internal reference and determine their exact masses. Meanwhile, it is not
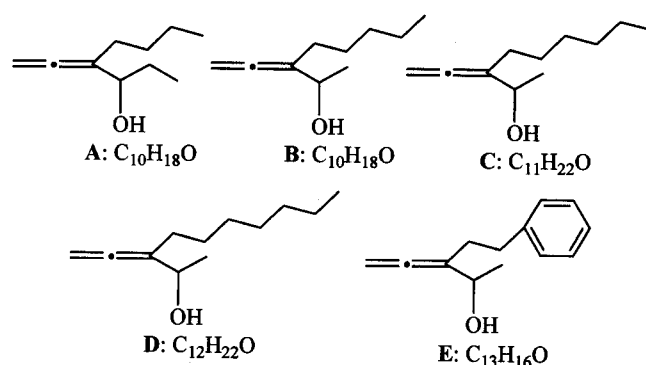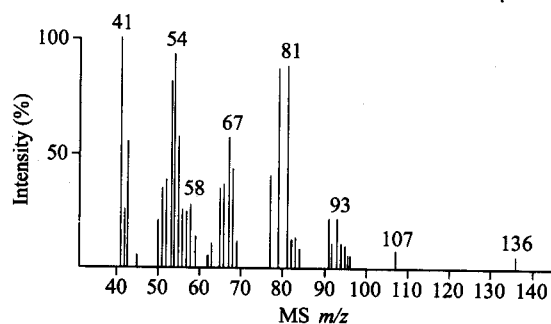

Fig. 2   EI mass spectrum of compound A ($M_W = 154$).

Fig. 3   CI mass spectrum of compound A ($M_W = 154$).

easy to explain the adduct ion peaks reasonably. CI is an unsuitable and unpractical method when coming to analyzing the exact mass of α-allenic alcohols. With the electrospray ionization (ESI), which is usually suitable to the strongly polar and thermally labile compounds such as peptides, proteins etc., we did not get the satisfied results from the corresponding mass spectrum either (Fig. 4). From the mass spectrum, the low intensity of the protonated molecular ion can be observed obviously. Meanwhile, for the same reason many existing adduct ion peaks made it difficult to select a proper internal reference to determine their exact protonated molecular ion MH^+ mass. So it is necessary to find an effective ionization method to solve this problem. Therefore atmospheric pressure chemical ionization (APCI) was finally tried. Compared with Figs. 4 and 5, it is not difficult to find that the sensitivity of APCI is clearly higher than that of ESI under the same concentration. Different from EI and other desorption/ionization methods, APCI is a 'softer' ionization method as good as ESI. The principle of this method can be described as follows: (1) it operates by an 'ion evaporation ionization' process, whereby ions are emitted from droplet into gas phase and ionized by a corona discharge at the discharge needle. (2) APCI leads to gas phase ionization of molecules and applies heat to the solvent flow to evaporate the high volume of liquid (typically used in LC/MS systems) to promote ionization of molecules where ESI conditions are not effective.[5] (3) To a certain degree like the self-chemical ionization (SCI),[6] APCI is also a kind of chemical ionization in which the reagent ions are fragment ions coming from the neutral reagent molecules (usually solvent molecules from the LC mobile phase). The solvent molecules ionized by a corona discharge produce a dry vapor of solvent and chemical ionization takes place in the atmospheric pressure. (4) The usual result is that the protonated molecular ions or other adducts are produced. Some unstable compounds such as α-allenic alcohol can get their adduct ion peaks $[M + H]^+$, $[MH + CH_3OH - H_2O]^+$, $[MH + CH_3OH - 2H_2O]^+$ easily with this 'soft' ionization technique (Fig. 5, methanol as solvent).[7] Thus we could determine the exact mass (or atomic compositions) of α-allenic alcohols by determining the exact mass of their corresponding adducts.
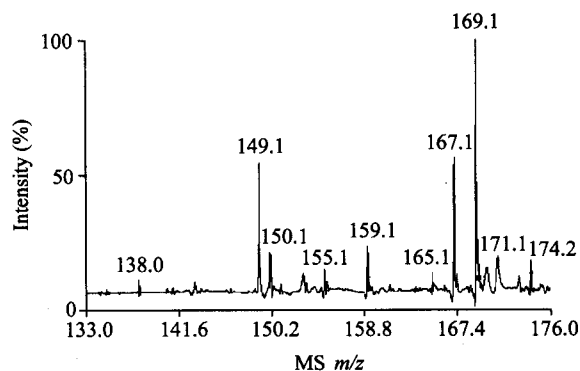

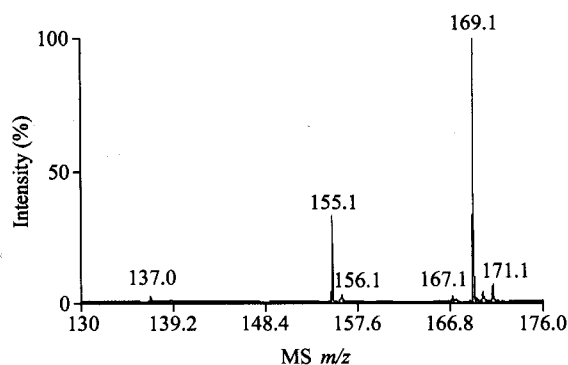Fig. 4 ESI mass spectrum of compound A ($M_W = 154$).

Fig. 5 APCI mass spectrum of compound A (methanol as solvent).

APCI is an analytical technique that is characterized by its simplicity, speed, sensitivity and wide range of applicability.[8-10] It is well known that chemical ionization rate and the efficiency of ionization will increase obviously for the special environment, the atmospheric pressure. Because the amount of internal energy imparted to generate ions needed by APCI is the lowest among all mass spectrometric ionization techniques except ESI, it always leads to an extremely low degree of fragmentation. Just like ESI/TOF-MS for the exact mass measurement,[11,12] APCI is also an optional ionization method for its mild operational condition when coming to analyze thermally labile compounds with relatively weak polarity.

Thus, APCI/TOF-MS was developed to determine the exact masses of five allenic alcohols via their protonated molecular ions using positive ion mode. In the positive ion mode, protonation is the usual ion/molecule reaction performed, the reagent ion transferring a proton to those analyte molecules presented in the sample vapor with a higher proton affinity. Polyethylene glycol (PEG) was used widely as the internal reference in ESI.[11,13,14] Because polyethylene glycol (PEG) has high sensitivity in APCI, it can be used as the internal reference. Samples were injected through a syringe pump with a constant flow rate to get a stable signal over a period of time and a better symmetric peak shape that affects the exact mass measurement. As a result, all the absolute errors compared with theoretical value were less than 1.0 mmu. Solvent effects were studied and the results showed that acetonitrile is a good solvent in our case. The effects of nozzle potential, push pulse potential, pull pulse potential, pull bias potential and acquisition rate on exact mass determination were also discussed.

Experimental

Chemicals

All reagents were of HPLC grade. PEG 200 was of analytical grade. All samples were synthesized by ourselves, and had been identified by ^1H NMR, ^{13}C NMR and IR spectra.[15]

Sample preparation

The sample solutions used in the exact mass determination were composed of PEG 200 and the compounds of interest both at the concentration of 0.08 mg/mL in methanol or acetonitrile. To obtain a good peak shape and similar signal intensity of the interest an appropriate volume ratio was also very important. Methanol or acetonitrile was used as mobile phase.

APCI/TOF-MS *conditions*

Experiment was performed on an Applied Biosystems Mariner time-of-flight mass spectrometer equipped with an atmospheric pressure chemical ionization ion source (ABI SCIEX Co. U.S.). Samples were introduced by a syringe pump with a flow rate of 20 μL/min. Mass spectra were recorded in the positive ion mode using the following conditions: corona discharge needle voltage was 4800 V and the nozzle potential was adjusted to 100 V unless otherwise stated. High purity nitrogen (99%) was used as nebulizing gas and curtain gas in APCI experiments and their flow rates were maintained at 0.4 and 2.0 L/min, respectively in all experiments. Detector voltage was 1850 V. Mass spectra were collected from m/z 100—300 with the acquisition rate of 3 s per scan. Resolving powers of about 5000 based on the full peak width at half peak height definition were routinely obtained and used in these experiments. The exact masses of m/z 151.09649, 195.12270, 239.14892, 283.17513 of PEG 200 were selected as the reference masses for external calibration (mass scale calibration).

Results and discussion

Effect of different solvents

The effect of different solvents on mass accuracy was studied. All results were obtained under the optimal conditions. The results showed that solvents had different effect on mass accuracy since the relative abundant of the protonated adducts of the samples were different in their mass spectra. When methanol acted as the solvent, the intensity of corresponding adduct $[MH + CH_3OH - H_2O]^+$ was higher than that of protonated molecular ions MH^+, which seriously affected the sensitivity of the expected MH^+ and finally affected the accuracy of exact mass measurement. Because a similar signal intensity of reference and analyte peaks is the key factor to the determination of the exact mass measurement.[7,11] However, when acetonitrile was chosen as the solvent, the corresponding mass spectra and the exact mass measurements with the reference mass of PEG 200 were shown in Fig. 6, there are a little other adducts except $[M + H]^+$. Due to the presence of trace methanol in our experiment, the adducted ion peaks $[MH + CH_3OH - H_2O]^+$ also appeared in the mass spectra. But compared with the protonated ions, the relative

abundant is very low. The different results obtained from two solvents are showed in Table 1. From Table 1, it can be seen that the solvent used in our experiment is very important and acetonitrile is better than methanol.
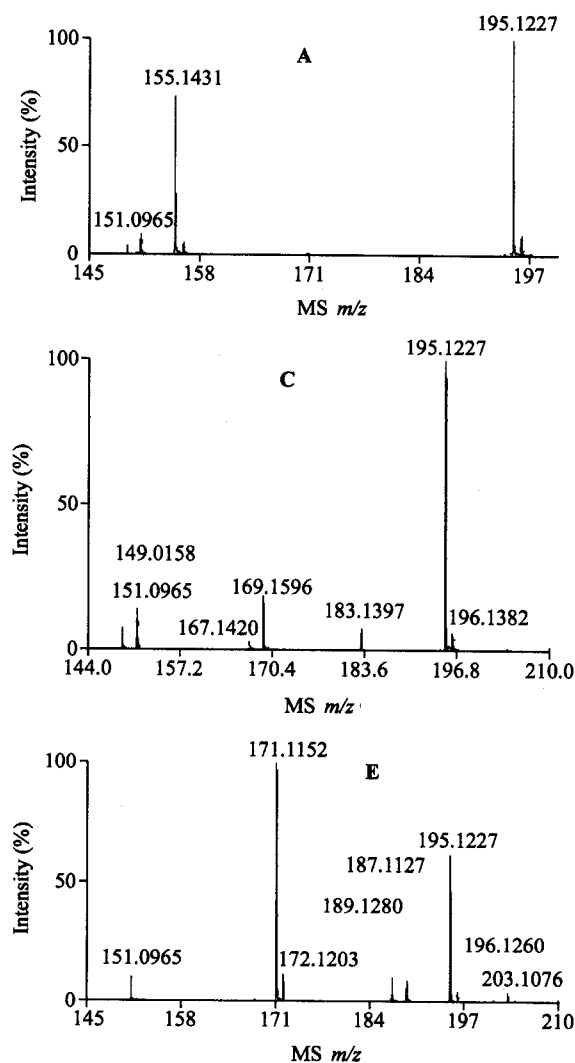
Fig. 6 Typical APCI mass spectra of compounds **A**, **C**, **E** and PEG 200 (in acetonitrile).

Table 1 Solvent effects on the exact mass measurement of MH^+

Compound	Absolute error (mmu)	
	Methanol	Acetonitrile
A	− 0.68	0.03
B	1.72	0.54
C	− 6.99	0.87
D	2.78	0.57
E	0.67	0.62

Preliminary condition of exact mass measurement

The following factors contribute to the accuracy of mass measurements: symmetry of peak shape, signal-to-noise ratio, similar signal intensity of reference and analyte peaks, two internal reference masses that bracket and close to the mass of interest.[7,11] The calibration of the in-

strument inspected the stability of the instrument and had a great influence on its sensitivity. It also verified whether the shapes of spectrum peaks appeare in Gaussian distribution. Because of its complex internal structure, it is necessary to have some skills to tune the optimal experimental condition for the stability of the APCI systems. In this process we acquired two standard peaks that ideally bracketed the mass range of interest. The seconds per spectrum score were set to 3 to obtain better peak statistics, $i. e.$, peak shape, signal-to-noise ratio, for calibration. In addition, the factors that affect peak shape and resolution during the exact mass measurement were also studied. In this section, compared with the theoretical values, all average deviations ($i. e.$ absolute errors) were less than 1.0 mmu. Exact masses of the five compounds were determined with two reference masses. The selected reference masses and the results are listed in Table 2.

Effect of nozzle potential

It is important to select an appropriate range of nozzle potential. Nozzle potential affects sensitivity, signal stability and fragmentation. A higher nozzle potential caused higher sensitivity and signal stability. For example we inspected compound **A** in detail under different nozzle potentials ranging from 20 to 150 V and found that when the nozzle potential reached a certain value the adduct ion peaks were hardly observed and the in-source CID happened. This indicated that fragmentation increased when the potential was above 100 V. As a result, nozzle potential of 100 V was selected. However under the same condition we almost can not see the protonated ion of the compound **E** and it is not facile to determine the exact mass of compound **E**. It is probably due to the fact that the molec-

ular polarity of compound **E** is stronger than that of the others. We tried to tune other parameters that affect sensitivity but failed, and only when the nozzle potential was adjusted to 10 V we did observe a satisfied result.

Effect of peak shape and resolution—optimal condition of exact mass measurement

The better peak shape and the resolution are important factors and major premises for the exact mass measurement. In our experiments there are two methods to improve the peak shape and the resolution. The key experimental parameters that affect the peak shape and the resolution are push pulse potential, pull pulse potential, pull bias potential which lie in the analyzer zone. Under the same conditions, acquisition rate also influence the peak statistics such as peak shape, signal-to-noise ratio, $etc.$ and these factors contribute to the accuracy of mass measurements. In the experiment it was found that in order to get a better peak shape and resolution, higher push and pull pulse potential, pull bias potential were needed with the lower nozzle potential. Higher acquisition rates caused better peak shape, resolution and better mass accuracy.

Conclusions

In summary, the atmospheric pressure chemical ionization (APCI)/time-of-flight mass spectrometry was successfully applied to determine the exact masses of five allenic alcohols via their protonated molecular ions. Acetonitrile was the proper solvent used in our experiment and satisfactory results were obtained. All absolute errors were less than 1.0 mmu. Some factors that affected the accuracy had to be taken into consideration.

Table 2 Exact mass measurements of MH$^+$ for the samples with two reference masses

Compound	Theoretical mass value (amu)	Reference		Average detected mass value (amu)	Absolute error (mmu)
		Low mass	High mass		
A	155.14304	151.09649	195.12270	155.14307	0.03
B	155.14304	151.09649	195.12270	155.14358	0.54
C	169.15869	151.09649	195.12270	169.15956	0.87
D	183.17434	151.09649	195.12270	183.17491	0.57
E^a	189.12739	151.09649	195.12270	189.12801	0.62

^{a} Nozzle potential: 10 V.

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