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Qualitative determination of an amide of unknown fragmentation pattern using Gas Chromatography – MassSpectrometry

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ABSTRACT:*Amide compounds are one of the main functional groups studied in supramolecular chemistry as hydrogen bonding sources. The synthesis of new amide compounds must always be supported by the characterization that proves the concordance between the theoretically designed product and the one that was experimentally obtained. Several spectroscopic techniques are usually employed for this purpose; however, gas chromatography coupled to mass spectrometry (CG - MS) is hardly used as a qualitative routine characterization technique of new compounds within this area because of the lack of reference mass spectra of similar compounds of known fragmentation pattern. In this study, a method for determining an amide containing aromatic and long alkoxy functional groups is proposed. The mass spectrum of the compound is presented and described. The comparison between the experimental ionization pattern and the theoretically expected primary ions reveals that the mass spectrum of the synthesized product may be used as a reference for identifying analog molecules, particularly, in the supramolecular chemistry field.*
KEYWORDS *amide, organic synthesis, gas chromatography, mass spectrometry.*

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I. INTRODUCTION

The amide group is a major organic functional group in chemistry since it is present in peptides, proteins, synthetic polymers, supramolecular units and other compounds which are of particular interest not only for chemistry but also for biology, physics, and materials science[1].

In supramolecular chemistry, amide compounds are matter of particular interest because the amide hydrogen bonding is considered as one of the most significant driving forces inducing the formation of supramolecular architectures[2]–[4], as a consequence of its inherent self-complementary hydrogen bond donor and acceptor ability[5].

Since the early beginnings of the XXI century, several amide compounds which include aromatic and long alkoxy functions have been demonstrated to be promising building blocks in supramolecular chemistry[6]–[11]. Currently, new amide compounds of similar structural characteristics are still under study for supramolecular applications; therefore, rapid, inexpensive, and efficient characterization of new products is mandatory.

Among the diverse methods which are typically available for the characterization of new organic compounds such as nuclear magnetic resonance of proton (^{1}H-NMR) or carbon-13 (^{13}C-NMR), Fourier transform infrared spectroscopy (FTIR), elemental analysis, and mass spectrometry (MS); gas chromatography coupled to mass spectrometry (GC-MS) attracts particular attention because it combines the separation capability of gas chromatography (GC) with the almost unequivocally identification power of mass spectrometry[12].

Although some organic substances of interest are frequently not suitable for being characterized through GC-MS because of their physicochemical properties, such as low volatility or high polarity, this is not necessarily true for amides. On the contrary, amides are commonly used as derivative substances that allow the

determination of amines, which are usually more challenging to extract and analyze than amides via CG-MS[13].

Several examples of amides and other nitrogenated compounds that have been quantitative determined through CG are found in literature, for instance, methamphetamine[14], residual amines in active pharmaceutical ingredients[15], amide derivatives of chiral 2-hydroxy acids[16], acrylamide[17], urea[18], and imide ionic liquids[19]. These quantifications have been possible because the signals in their corresponding chromatograms are assumed to qualitatively agree to the chemical species of interest, given that the mass spectrum of the sample matches with a reference spectrum.

Nevertheless, compounds that have been synthesized for the first time are not easily identified through GC-MS. For instance, in the field of supramolecular chemistry, amide compounds are not usually qualitatively characterized by means of this technique even though it could be used as a routine, fast, and high-sensitive method. Since the pattern and relative abundance of fragments in the mass spectrum are unique for a specific compound, the analyte could be recognized by comparing the experimental mass spectrum with those collected in electronic databases if the spectrum of the analyte has been previously recorded[20]; however, if no reference mass spectra are available for comparison, the elucidation of the compound becomes challenging.

In this study, a GC-MS method was developed for recording the mass spectrum of a new amide compound, the 4-(11-hexylcarbamoyl-undecanoxy)-benzoic acid methyl ester,which is used as building block for supramolecular aggregates formation. The chemical structure of this compound is shown in Fig. 1. GC-MS characterization was used for revealing the formation of the ions in which the compound is typically fragmented under the analysis conditions, which is a piece of evidence that indicates that the designed molecule wasfavorably obtained through the proposed reaction route[21].

The presence of the theoretically expected structural fragments in the mass spectrum of the amide confirmed that the experimental mass spectrumcorresponds to the mass spectral fragmentation pattern of the designed molecule and may be used as a reference for future research on similar compounds.

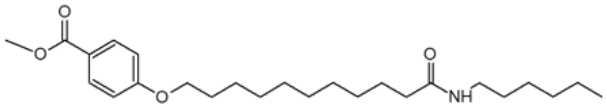

Figure 1. Chemical structures of 4-(11-hexylcarbamoyl-undecanoxy)-benzoic acid methyl ester

II. MATERIALS AND METHODS

2.1 Materials

Tetrabutylammonium bromide (95%), potassium carbonate (99%), 4-hydroxybenzoic acid methyl ester (≥99%), and dimethylformamide(99.7%)were purchased from Sigma-Aldrich; hydrochloric acid (37%) was purchased from Emsure.

2.2 Synthesis

4-(11-hexylcarbamoyl-undecanoxy)-benzoic acid methyl ester

The compound was prepared through a Williamson etherification synthesis based on a published methodology [10]. The synthesis consisted in dissolving 4-hydroxybenzoic acid methyl ester (363 mg, 2.4 mmol), 11-bromoundecanoic acid hexylamide(913 mg, 2.6 mmol), potassium carbonate (659 mg, 4.8 mmol), and tetrabutylammonium bromide (Bu_4NBr, 154 mg, 0.5 mmol) in N,N-dimethylformamide (DMF, 90 mL) under an inert nitrogen atmosphere. The mixture was stirred at 700 rpm and heated at 50 ± 5 °C for 12 h. Subsequently, the reactive mixture was poured into deionized water (300 mL) and the pH of the solution was adjusted to 1-2 using aqueous HCl (10% w/w), which resulted in the precipitation of a white solid that was vacuum filtered and used without further purification (710 mg, yield = 71%).

2.3 Gas Chromatography-Mass Spectrometry (GC-MS)

The GC-MS analysis was performed in a Perkin Elmer GC-MS Clarus 600 system equipped with a non-polar 5% phenyl 95% dimethyl polysiloxane cross-bonded column. The mass spectrometer was set for electron impact at 70 eV. The m/z ratio ranged from 29 to 500. The chromatographic program used was 50 °C → 6 °C/min → 200 °C → 20 °C/min → 350 °C. The sample was dissolved in methanol at a concentration of 100 ppm.

III. RESULTS AND DISCUSSIÓN

At the experimental conditions detailed in section II, the chromatogram of 4-(11-hexylcarbamoyl-undecanoxy)-benzoic acid methyl ester exhibited a unique chromatographic peak at a retention time (RT) of 34.72 s, as shown in Fig. 2. Additionally, the chromatograms of the starting reagents involved in the synthesis are presented in the same figure. The signals of both 4-hydroxybenzoic acid methyl ester (Fig. 2a)and 11-bromoundecanoic acid hexylamide(Fig. 2b) were not detected in the chromatogram of the amide product (Fig. 2c), which implies that any unreacted species remained in the final compound and suggests that it was obtained essentially pure.

Furthermore, the retention times agree with the expected elution sequence according to the molecular mass (MW) of the components, since 4-hydroxybenzoic acid methyl ester (RT = 15.80 s, MW = 152.15 Da) is lighter than both 11-bromoundecanoic acid hexylamide(RT = 24.73 s, MW = 348.36 Da) and the final amide (RT = 34.72 s, MW = 419.60 Da).

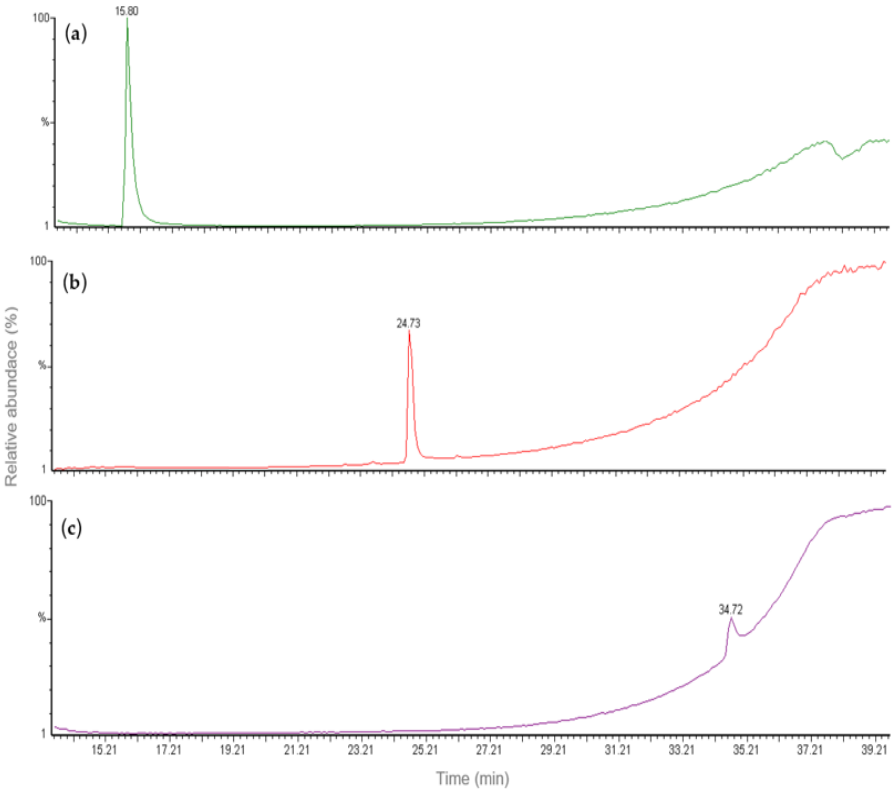

Figure 2. Chromatograms of (a) 4-hydroxybenzoic acid methyl ester, (b) 11-bromoundecanoic acid hexylamide, (c) 4-(11-hexylcarbamoyl-undecanoxy)-benzoic acid methyl ester

The chromatographic peak of RT = 34.72 s of Fig. 2(c)—attributed to the amide product—was further analyzed by obtaining its mass spectrum, in order to validate its identification. The mass spectrum of 4-(11-hexylcarbamoyl-undecanoxy)-benzoic acid methyl ester is shown below, in Fig. 3.

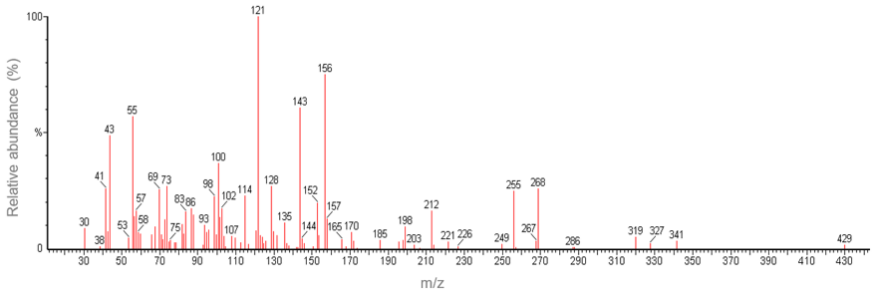

Figure 3. Mass spectrum of 4-(11-hexylcarbamoyl-undecanoxy)-benzoic acid methyl ester

Fig. 3 shows the mass spectrum of the amide product. From the 10 most abundant fragment signals appearing in Fig. 3, the one of highest intensity (m/z = 121) comes from one of the most abundant ions of the

reagent 4-hydroxybenzoic acid methyl ester, which is of known fragmentation pattern, available in the NIST library, 2018[22], while other seven are linked to the main fragments the other reagent, 11-bromoundecanoic acid hexylamide (m/z = 43, 55, 73, 128, 143, 156, 268).

In particular, the ions of m/z = 43, 55, and 73, are some of the most abundant fragments appearing in the mass spectra of 11-bromoundecanoic acid and 1-hexanamine[22]. The signal of Fig. 3 having a relation m/z = 143comes from the loss of a methylene group and protonation of the ion $[C_9H_{18}NO]^{+\cdot}$, which was produced by the fragmentation of the C–C bond of the β atom to the carbonyl group of the amide[23], while the fragment $[C_9H_{18}NO]^{+\cdot}$ by itself (m/z = 156) is also one of the most intense signals. The signal of relation m/z = 268 corresponds to the ion $[C_{17}H_{34}NO]^{+\cdot}$ that was generated by the elimination of the bromide ion from the 11-bromoundecanoic acid hexylamide molecule, which is consistent with the existence of fragments having m/z relations close to the atomic mass of bromine.

In addition to the ions related to the fragmentation of its starting reagents, four fragments of low intensity account for the aromatic ether function of the amide product: the fragment $[C_8H_7O_3]^{+\cdot}$ of m/z = 151, is explained by the rupture of the aliphatic C–O bond of the oxo-amide chain attached to the aromatic ring through the ether bond, while the ions $[C_8H_7O_2]^{+\cdot}$ and $[C_{17}H_{34}NO_2]^{+\cdot}$ of m/z = 135 and 284, respectively, are attributed to the rupture of the C–O bonding between the aromatic carbon of the ring that is joined to the oxygen to form the aromatic ether[23].

IV. CONCLUSION

Even though CG-MS characterization is a common quantitative technique for determination of organic compounds due to its high resolution, sensitivity, reproducibility, and short analysis times, the lack of reference fragmentation patterns of first-time synthesized compounds makes unable the qualitative identification of new products by comparison with reference mass spectra. A set of experimental conditions was proposed in this paper for recording the mass spectrum of a new amide product, the 4-(11-hexylcarbamoyl-undecanoxy)-benzoic acid methyl ester. The experimental conditions allowed the volatilization of the sample consisting of a methanol/amide solution while preventing the decomposition of the compounds. The chromatograms of the samples revealed single chromatographic peaks at retention times that are coherent with the expected elution sequence of the product in comparison with the starting reagents. Moreover, the experimental fragmentation pattern of the product is consistent with the theoretically expected ions and confirms the results of complementary spectroscopic characterization techniques. Therefore, the mass spectrum of the amide product may be used as a reference MS ionization pattern for the rapid and efficient identification of analog compounds having similar functional groups and molecular weights.

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