Vol. 11, No. 1, 2020
ISSN 2233-4203/ e-ISSN 2093-8950
ARTICLE www.msletters.org | Mass Spectrometry Letters

Characterization of an Unconventional MALDI-MS Peak from DHB/pyridine Ionic Liquid Matrices

Jangmi Hong and Jeongkwon Kim*

Department of Chemistry, Chungnam National University, Daejeon 34134, Republic of Korea

Received March 13, 2020, Revised March 20, 2020, Accepted March 23, 2020
First published on the web March 31, 2020; DOI: 10.5478/MSL.2020.11.1.6

Abstract : Matrix-assisted laser desorption/ionization-mass spectrometry (MALDI-MS) analysis of ionic liquid matrices (ILMs) prepared using pyridine and dihydroxybenzoic acid (DHB), such as 2,3-DHB and 2,5-DHB, displayed an unconventional peak at *m/z* 232.0, which was regarded as $[DHB+pyridine-H]^+$. The peak at *m/z* 232.0 was not observed from other ILMs prepared using other DHB isomers, such as 2,4-DHB, 2,6-DHB, 3,4-DHB, and 3,5-DHB. Two requirements to observe the peak at *m/z* 232.0 in a DHB/pyridine ILM are suggested. First, carboxyl and hydroxyl groups must be located *ortho* to each other. Second, the secondary hydroxyl group must be located at a carbon with a high electron density. Based on these two requirements, a potential mechanism for the generation of the peak at *m/z* 232.0 is suggested.

Keywords : Dihydroxybenzoic acid, pyridine, ionic liquid, ionic liquid matrix, MALDI

Introduction

Matrix-assisted laser desorption/ionization-mass spectrometry (MALDI-MS) is a powerful analytical tool. Selection of a suitable matrix is an important aspect of MALDI-MS analysis because each matrix displays distinct efficiencies for specific analytes. Various matrices are currently used for the analysis of samples by MALDI-MS.[1] These include organic matrices such as dihydroxybenzoic acid (DHB), α-cyano-4-hydroxycinnamic acid, and sinapinic acid.

The formation of heterogeneous hot spots comprises a serious problem involved in the use of convectional organic matrices; these hot spots result in uneven sample distribution. Therefore, it becomes difficult to quantify an analyte using a conventional acid matrix. To overcome this issue, various ionic liquids have been introduced as MALDI matrices.[2-4] Ionic liquids are characterized by their nonvolatility, high thermal stability, and good solubility. Notably, ionic liquid matrices (ILMs) enable homogeneous sample distribution, which aids in MALDI-MS quantification. A commonly used ILM comprises a mixture of 2,5-DHB and pyridine.[5-7] We observed an unexpected peak at *m/z* 232.0 when we used 2,5-DHB/pyridine as the ILM. In the present study, various ILMs composed of pyridine with DHBs (e.g., 2,3-DHB, 2,4-DHB, 2,5-DHB, 2,6-DHB, 3,4-DHB, and 3,5-DHB) were tested to characterize this peak; we confirmed that the peak was only observed for ILMs composed of 2,3-DHB/pyridine and 2,5-DHB/pyridine. In addition, we provide a potential mechanism for the generation of the peak at *m/z* 232.0.

*Reprint requests to Jeongkwon Kim
E-mail: jkkim48105@cnu.ac.kr

Experimental

All of the chemicals in this study (i.e., 2,3-DHB, 2,4-DHB, 2,5-DHB, 2,6-DHB, 3,4-DHB, 3,5-DHB, pyridine, methanol, ethanol, acetonitrile, and trifluoroacetic acid) were purchased from Sigma-Aldrich (St. Louis, MO, USA). To prepare ILM solutions, the following steps were used: DHB solutions were first prepared by dissolution of 1 mol of 2,3-DHB, 2,4-DHB, 2,5-DHB, 2,6-DHB, 3,4-DHB, or 3,5-DHB in 0.5 mL of pure methanol. To each DHB solution, solid pyridine was then added at a molar ratio of 1:1. Solutions were then mixed for 30 min, completely dried, and reconstituted by addition of 0.5 mL of 100% ethanol. The MALDI mass spectra for the DHB solutions were acquired using an Axima CFR MALDI-TOF-MS (Shimadzu Biotech, Tokyo, Japan) equipped with a 337-nm N_2 laser in positive-ion reflectron mode. The MALDI mass spectra for the DHB/pyridine ionic liquid samples were obtained using a MALDI-TOF-MS (Voyager DE-STR; Applied Biosystems, Foster City, CA, USA) at

the CNU Chemistry Core Facility (Daejeon, South Korea) equipped with a 337-nm N_2 laser in positive-ion reflectron mode.

Results and Discussion

Various commercially available DHB isomers (i.e., 2,3-DHB, 2,4-DHB, 2,5-DHB, 2,6-DHB, 3,4-DHB, and 3,5-DHB) were inv estigated to establish which DHB isomer could produce the peak at *m/z* 232.0 in the MALDI-MS analysis of the ILM of DHB/pyridine. Figure 1 shows the MALDI mass spectra of individual DHB isomers. All DHB isomers displayed a peak at *m/z* 137.0 $[DHB\text{-}OH]^-$. When used as a matrix, the DHB isomer should contain an intramolecular hydrogen bond.[8,9] This requirement is satisfied by 2,3-DHB, 2,4-DHB, 2,5-DHB, and 2,6-DHB, which all showed DHB derivative peaks at *m/z* 137.0 [DHB-OH or $C_7H_5O_3]^+$, 154.0 [DHB• or $C_7H_6O_4]^{+\bullet}$, 155.0 [DHB+H or $C_7H_7O_4]^+$, 177.0 [DHB+Na or $C_7H_6O_4Na]^+$, 199.0 [DHB-H+2Na or $C_7H_6O_4Na_2]^+$, and 273.1 [2DHB-H_2O-OH or $C_{14}H_9O_6]^+$.[10]

Figure 2 shows the MALDI mass spectra of the ILMs where each MALDI spot was prepared by combining one DHB isomer and pyridine. The images of all ILM spots look similar. A pyridine peak at *m/z* 80.0 [pyridine+H or $C_5H_6N]^+$ was always observed. Several common DHB-derivative peaks at *m/z* 137.0, *m/z* 177.0, and *m/z* 199.0 were also observed for the ILMs of pyridine mixed with 2,3-DHB, 2,4-DHB, or 2,5-DHB. A dominant peak at *m/z* 232.0 was observed for the ILMs of 2,3-DHB/pyridine and 2,5-DHB/pyridine. Considering the monoisotopic masses of DHB (154.0) and pyridine (79.0), we presumed that the peak at *m/z* 232.0 originated from DHB+pyridine-H, which is not a common formula for a positive-ion mode mass spectrum.

Figure 3 shows the potential mechanism for the formation of the *m/z* 232.0 ion from 2,5-DHB and pyridine. In step 1, protonation of pyridine and deprotonation of 2,5-DHB occur, along with intramolecular proton transfer between the *ortho* carboxylic and hydroxyl groups. Intramolecular proton transfer in a DHB matrix isomer is necessary for a MALDI matrix.[8,9] In step 2, nucleophilic aromatic substitution occurs, in which the oxygen anion in the DHB ion acts as a nucleophile, attacking the electron-poor carbon atom in pyridine. This substitution is possible because the carbon atom next to the nitrogen atom in a pyridine has the lowest electron density.[11] The reaction is similar to the Chichibabin reaction,[12] although the oxide anion acts as the nucleophile, while the amide anion does not. In step 3, hydride subtraction occurs, which allows resonance structures of the compounds. The carboxylic group in a DHB isomer acts as an electron-withdrawing group, which results in an electron-poor environment for C-2, C-4, and C-6. Because the hydroxyl group is an electron-donating group, the hydroxyl group at C-2 generates an electron-rich environment for C-1, C-3, and C-5. Therefore, the hydroxyl group attached to C-3 or C-5 of 2-hydroxybenzoic acid (2,3-DHB or 2,5-DHB) is electron-rich; it can easily participate in nucleophilic aromatic substitution.

Based on the above mechanism, several aspects of a DHB isomer in a DHB/pyridine ILM explain the presence of the peak at *m/z* 232.0. First, the carboxyl and hydroxyl groups in a DHB must be located *ortho* to each other to

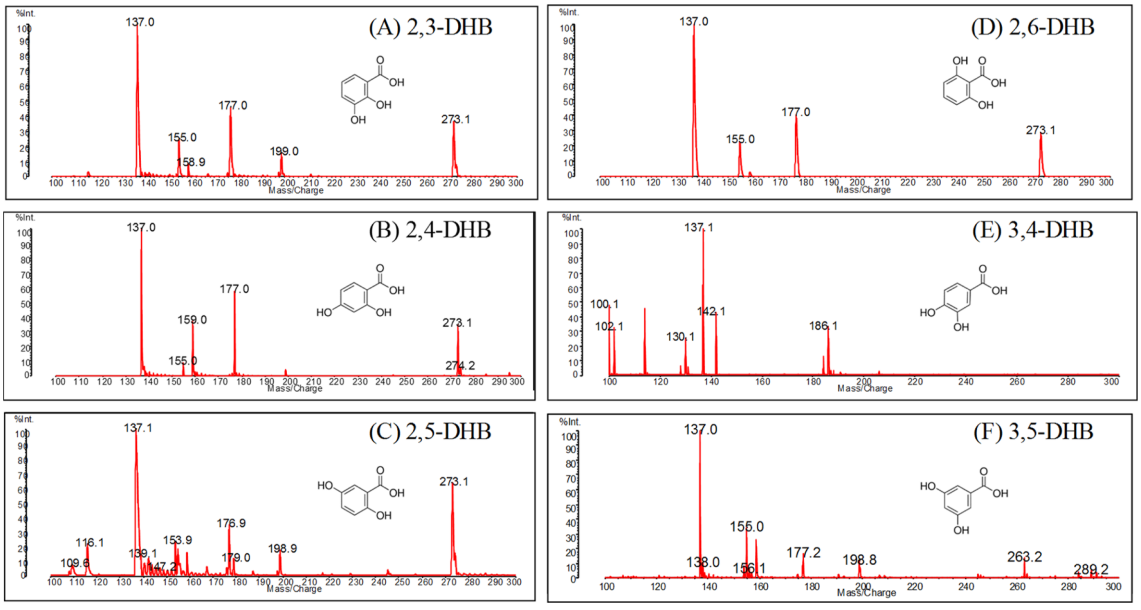

Figure 1. MALDI mass spectra of (A) 2,3-DHB, (B) 2,4-DHB, (C) 2,5-DHB, (D) 2,6-DHB, (E) 3,4-DHB, and (F) 3,5-DHB.

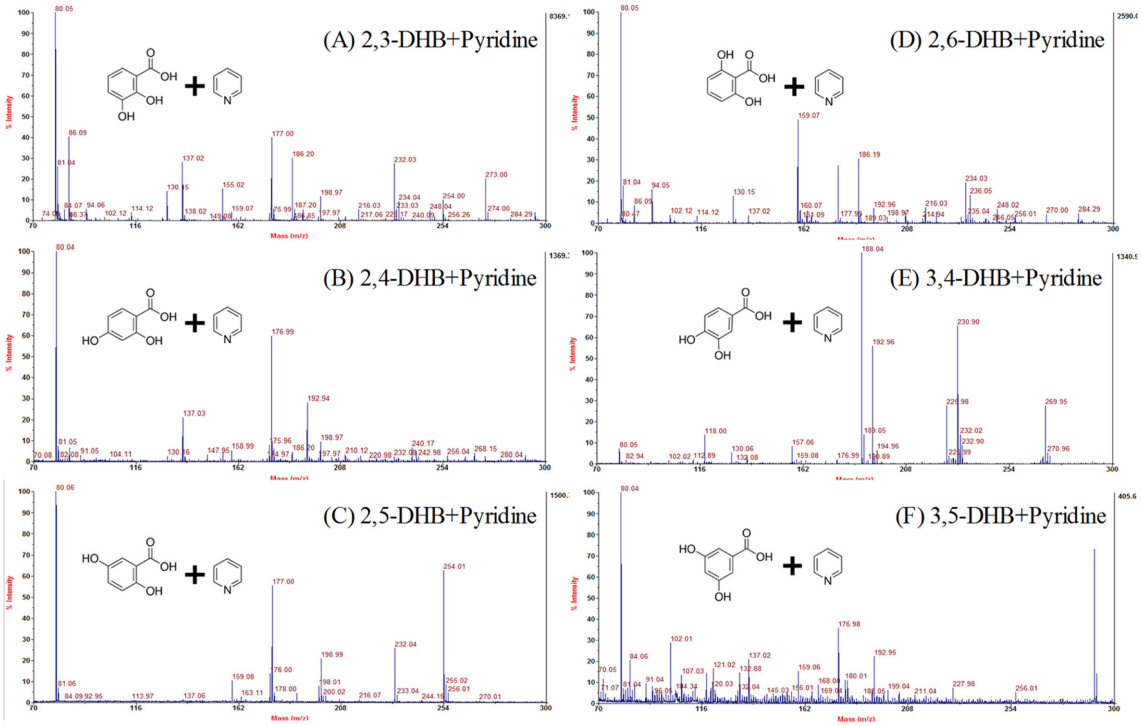

Figure 2. MALDI mass spectra of ionic liquid matrices containing (A) 2,3-DHB+pyridine, (B) 2,4-DHB+pyridine, (C) 2,5-DHB+pyridine, (D) 2,6-DHB+pyridine, (E) 3,4-DHB+pyridine, and (F) 3,5-DHB+pyridine.

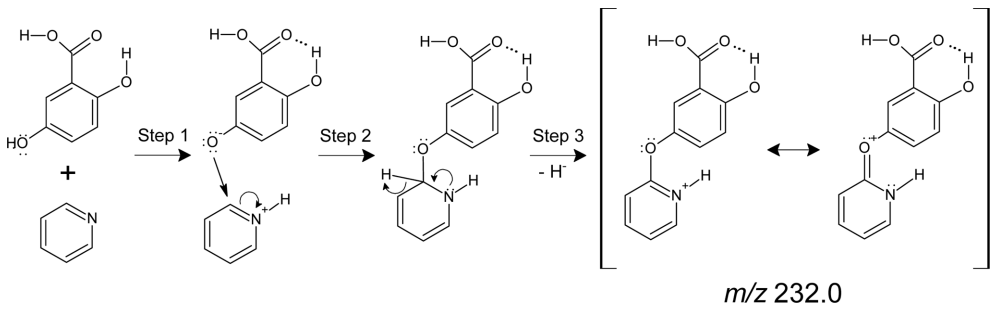

Figure 3. Mechanism showing formation of the *m/z* 232.0 ion from 2,5-DHB and pyridine.

form an intramolecular hydrogen bond. Second, the secondary hydroxyl group must be located at a carbon with a high electron density, such as *ortho* or *para* to the first hydroxyl group or *meta* to the carboxyl group. Because only 2,3-DHB and 2,5-DHB satisfy these requirements, their ILMs with pyridine produce the peak at *m/z* 232.0.

Conclusions

The results of this study suggest a mechanism for generation of an unconventional peak at *m/z* 232.0, regarded as $[DHB+pyridine-H]^+$ in the MALDI-MS analysis of the ILMs of pyridine with 2,3-DHB and 2,5-DHB. The mechanism involves protonation of pyridine, deprotonation of a DHB isomer, nucleophilic aromatic substitution, and hydride-ion subtraction. The resonance structures of the compounds are presumed to contribute to stabilization of the compound. The peak could serve as a calibrant in MALDI-MS analysis using an ILM composed of pyridine and 2,5-DHB.

Acknowledgements

We acknowledge the generous help from the CNU Chemistry Core Facility. This work was supported by research funding from Chungnam National University.

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