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Competing benzyl cation transfers in the gas-phase fragmentation of the protonated benzyl phenylalaninates

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ABSTRACT

In this study, the competing benzyl cation transfer reactions have been explored by investigating the gas phase chemistry of the protonated benzyl phenylalaninates. Protonation at the carboxylic O atom results in the breakage of the ester C—O bond to afford the benzyl cation, which undergoes the competing migration to the amino N atom or the phenyl ring C atom. Both the amino and the phenyl ring hydrogen atoms can be activated to be mobile due to the electrophilic attack of the transferring benzyl cation, and migration of the activated hydrogen atom to the carboxylic hydroxyl leads to (H_2O+CO) elimination of the precursor ion. Interestingly, it is much more preferred for the benzyl cation to transfer to the phenyl ring via the amino N, leading to the stepwise benzyl cation transfer, albeit the amino N atom contains more nucleophilic affinity. The mechanistic processes have been confirmed by the MS^3 spectra data, along with D-labeling experiments and theoretical calculations.

1. Introduction

Since the introduction by Yamashita and Fenn in 1984 [1], electrospray ionization mass spectrometry (ESI-MS) has been widely applied in analyzing a large variety of compounds [2–6]. In particular, collision-induced dissociation (CID) enjoys its unique advantages in structure elucidation by providing abundant fragmentation data [4,5]. However, the MS-based structural elucidation has also been challenged by the widespread unexpected rearrangement reactions, such as the benzyl cation transfer [7–15], occurring in the fragmentation process, and these reactions have attracted great interest among analysts since the early days of organic mass spectrometry.

Benzyl cation is a highly reactive intermediate in various chemical and biochemical reactions [16]. Fragmentation of the protonated benzylated derivatives is facile to afford the benzylium-contained ion/neutral complex (INC). Besides the direct separation to form benzyl cation, many interesting product ions have been generated in mass spectrometry via the INC-mediated reactions, such as electrophilic aromatic substitution [7–13], hydride transfer [17], electron transfer [18] and nucleophilic aromatic substitution [14,15]. Benzyl cation has been previously reported to migrate to the phenyl ring mediated by the benzylium-contained INC [7–15]. In our previous work, benzyl cation has been found to be directly transferred to the amino nitrogen before dissociation of the protonated benzyl prolinates occurs [19]. Phenylalanine possesses both amino nitrogen and phenyl ring, both of which can accept the electrophilic attack of the transferring benzyl cation. With this in mind, benzyl phenylalaninate was selected as a model molecule in this work to extend the mechanistic investigation on the competing benzyl cation transfers.

2. Experimental

2.1. Materials

O-Benzylated phenylalanine derivatives (compounds **1**–**7** in Scheme 1) were synthesized according to the classical method, involving reaction of *Boc*-protected phenylalanine with the corresponding benzyl chloride in the presence of Cs_2CO_3 [20]. The subsequent deprotection of *Boc* is carried out in the presence of TFA–DCM (V_{TFA}:V_{DCM} = 1:1) to obtain the target compounds [21]. The *N*-benzylated phenylalanine derivative (compound **8**) was obtained from the reaction of phenylalanine with benzyl chloride in the presence of K_2CO_3 in water, according to a procedure described in the literature [22]. *N*-benzylated 2-phenylethanimine

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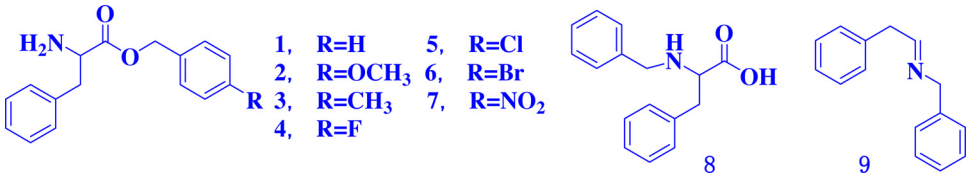

Scheme 1. Structures of substituted benzyl phenylalaninates (**1**–**7**), *N*-benzylated phenylalaninate (**8**) and *N*-benzylated 2-phenylethanimine (**9**).

(compound **9**) was synthesized by phenylacetaldehyde and benzylamine in the presence of $MgSO_4$ in ethanol solution. All compounds were purified after synthesis, and their structures were further confirmed by ^1H NMR, ^{13}C NMR and MS.

2.2. Mass spectrometry

The ESI-MS/MS experiments were performed on an LCQ advantage mass spectrometer (Thermo Fisher Company, USA), equipped with an ESI ion source in the positive ionization mode, with data acquisition using the Xcalibur software (Version 1.4). Typical parameters for the operation of the ESI-MS were used as previously described [19].

Accurate MS of the product ions were measured by a micrOTOF-QII (Q-TOF) mass spectrometer (Bruker Company, USA), equipped with an ESI ion source. The collision energy of the CID for the selected ions was set at 8 eV with Argon being used as the collision gas. The instrument was operated at a resolution higher than 15,000 full width at half maximum at *m*/*z* 922 using the micrOTOF-Q control program (Version 2.3). The data were analyzed using the Data Analysis Version 4 software package delivered by Bruker Daltonics.

2.3. Theoretical calculations

The theoretical calculations were performed using the Gaussian 03 program [23]. The structures of the reactants, transition states, intermediates and products were optimized using the density functional theory (DFT) method at the B3LYP/6-31 + G(2d,p) level. All reactants, intermediates and products were identified as the true minima by the absence of imaginary frequencies. Transition states, on the other hand, were identified by the presence of one single imaginary vibration frequency and the normal vibrational mode. The transition states were further confirmed using the intrinsic reaction coordinates calculations. The energies discussed here are the sum of the associated electronic and thermal free energies. The DFT-optimized structures were shown by GaussView (Version 3.09) software to give higher quality images of these structures. Hard data on geometries of all the structures considered are available in the Supplementary material.

3. Results and discussion

3.1. Dissociation of the protonated benzyl phenylalaninates

Investigation of the competing benzyl cation transfer reactions have been carried out by exploring the fragmentation behaviors of the protonated substituted benzyl phenylalaninates (Table 1). Benzyl phenylalaninate (**1**) was selected as a model to perform a detail investigation. Fig. 1(a) describes the CID-MS spectrum of $[\mathbf{1}+H]^+$, in which the fragment ion at *m*/*z* 91 is the benzyl cation, originating from the direct decomposition of $[\mathbf{1}+H]^+$. Ammonia elimination of $[\mathbf{1}+H]^+$ leads to the fragment ion at *m*/*z* 239, which subsequently undergoes the H_2O elimination to give the product ion at *m*/*z* 221. The simultaneous (H_2O+CO) expulsion of $[\mathbf{1}+H]^+$ occurs to afford the most abundant ion at *m*/*z* 210 in the CID-MS spectrum via the benzyl cation transfer [19,24]. The subsequent ammonia elimination of the ion at *m*/*z* 210 results in the product ion at *m*/*z* 193. Also, the product ion at *m*/*z* 120 is assigned to the 2-phenylethaniminium, resulting from the simultaneous loss of ($PhCH_2OH+CO$). The elemental compositions of these fragment ions have been determined by analyzing their accurate masses using high resolution Q-TOF mass spectrometer (Supplementary Table 1S and Fig. 1S).

All of the protonated substituted benzyl phenylalaninates show similar fragmentation behaviors, indicating a much favorable dissociation channel of losing (H_2O+CO) via benzyl cation transfer (Table 1 and Supplementary Fig. 2S). The favorable reaction of (H_2O+CO) elimination was also consolidated by investigating the breakdown curves at various collisional energies (Supplementary Fig. 3S).

Two sites (the amino nitrogen atom and the A ring carbon atom) in the structure of phenylalanine was found to potentially accept the electrophilic attack of the transferring benzyl cation, and the potential reaction channels of the competing benzyl cation transfer are proposed in Scheme 2. In path-a, the benzyl (B ring) cation is transferred from the ester oxygen atom O4 to the amino

Table 1
The CID MS data of the protonated phenylalanine substituted-benzyl esters in Scheme 1 at the normalized collision energy (NCE) of 24%.

Compound	R	$[M+H]^+$ *m*/*z* (%)	Product ions, *m*/*z* (%)					
			Losing ($CO+H_2O$) ion **a** or **b**	$[\mathbf{a}-NH_3]^+$ or $[\mathbf{b}-NH_3]^+$	Losing NH_3 ion **c**	$[\mathbf{c}-H_2O]^+$	Losing ($CO+PhCH_2OH$) ion **d**	R-$C_7H_6^+$
1	—H	256 (43.3)	210 (100%)	193 (21.3%)	239 (15.4%)	221 (9.8%)	120 (4.5%)	91 (23.0%)
2	—OCH_3	286 (7.6)	–	–	–	–	120 (0.8%)	121 (100%)
3	—CH_3	270 (21.7)	224 (13.4%)	207 (1.7%)	253 (0.8%)	235 (0.9%)	120 (0.1%)	105 (100%)
4	—F	274 (33.0)	228 (94.2%)	211 (8.3%)	257 (14.4%)	239 (3.0%)	120 (1.6%)	109 (100%)
5	—^{35}Cl	290 (73.3)	244 (91.1%)	227 (7.1%)	273 (13.4%)	255 (2.2%)	120 (1.7%)	125 (100%)
	—^{37}Cl	292 (67.6)	246 (94.5%)	229 (9.4%)	275 (14.6%)	257 (3.2%)	120 (2.0%)	127 (100%)
6	—^{79}Br	334 (35.6)	288 (100%)	271 (8.1%)	317 (11.6%)	299 (2.7%)	120 (1.2%)	169 (88.7%)
	—^{81}Br	336 (33.9)	290 (100%)	273 (9.4%)	319 (10.9%)	301 (2.4%)	120 (1.1%)	171 (94.5%)
7	—NO_2	301 (100)	255 (51.4%)	238 (22.1%)	284 (10.6%)	266 (7.6%)	120 (40.6%)	136 (<0.1%)