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An unprecedented rearrangement in collision-induced mass spectrometric fragmentation of protonated benzylamines

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The collision-induced dissociation (CID) mass spectra of several protonated benzylamines are described and mechanistically rationalized. Under collision-induced decomposition conditions, protonated dibenzylamine, for example, loses ammonia, thereby forming an ion of *m/z* 181. Deuterium labeling experiments confirmed that the additional proton transferred to the nitrogen atom during this loss of ammonia comes from the *ortho* positions of the phenyl rings and not from the benzylic methylene groups. A mechanism based on an initial elongation of a C–N bond at the charge center that eventually cleaves the C–N bond to form an ion/neutral complex of benzyl cation and benzylamine is proposed to rationalize the results. The complex then proceeds to dissociate in several different ways: (1) a direct dissociation to yield a benzyl cation observed at *m/z* 91; (2) an electrophilic attack by the benzyl cation within the complex on the phenyl ring of the benzylamine to remove a pair of electrons from the aromatic sextet to form an arenium ion, which either donates a ring proton (or deuteron when present) to the amino group forming a protonated amine, which undergoes a charge-driven heterolytic cleavage to eliminate ammonia (or benzylamine) forming a benzylbenzyl cation observed at *m/z* 181, or undergoes a charge-driven heterolytic cleavage to eliminate diphenylmethane and an immonium ion; and (3) a hydride abstraction from a methylene group of the neutral benzylamine to the benzylic cation to eliminate toluene and form a substituted immonium ion. Corresponding benzylamine and dibenzylamine losses observed in the spectra of protonated tribenzylamine and tetrabenzyl ammonium ion, respectively, indicate that the postulated mechanism can be widely applied. The postulated mechanisms enabled proper prediction of mass spectral fragments expected from protonated butenafine, an antifungal drug. Copyright © 2006 John Wiley & Sons, Ltd.

KEYWORDS: hydrogen transfer; fragmentation; CID; benzylamines; deuterium-labeled compounds; butenafine; ion/neutral complex

INTRODUCTION

Many benzylamines are biologically important compounds. For example, several neurotransmitters such as catecholamines[1–3] and drugs such as butenafine (*N*-4-*tert*-butylbenzyl-*N*-methyl-1-naphthalenemethylamine),[4] terbinafine,[4] and ambenonium chloride[5] are benzylamine derivatives. Mass spectrometry in conjunction with electrospray ionization and collision-induced dissociation (CID) shows promise in the prognosis of various diseases and in the identification of metabolic by-products. Recently, selected reaction monitoring (SRM) techniques have been employed for the identification and quantification of norepinephrine and catecholamine.[1] Success of all these analytical procedures depends on conceptual understanding of fragmentation mechanisms.

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Although odd-electron ion fragmentations (electron-ionization mass spectrometry) have been widely examined and rules established, we know less about even-electron fragmentation and rearrangement mechanisms. In our quest for understanding the intricate rearrangement mechanisms involved in the fragmentation processes of even-electron ions, we investigated the spectra of several benzylamines (Fig. 1), and we present our results here.

EXPERIMENTAL

Mass spectrometry

The CID mass spectra were recorded on a Micromass Quattro 1 spectrometer equipped with an electrospray source. Samples were introduced to the source as acetonitrile–water–formic acid ($9:1:10^{-2}$) solutions for positive-ion MS and as acetonitrile–water–ammonia ($9:1:10^{-2}$) solutions for negative-ion MS at a flow rate of 320 µl h^{-1}. The source temperature was held constant at 80 °C. The argon gas pressure in the collision cell was set to attenuate precursor ion transmission by 30–50%. For experiments performed in

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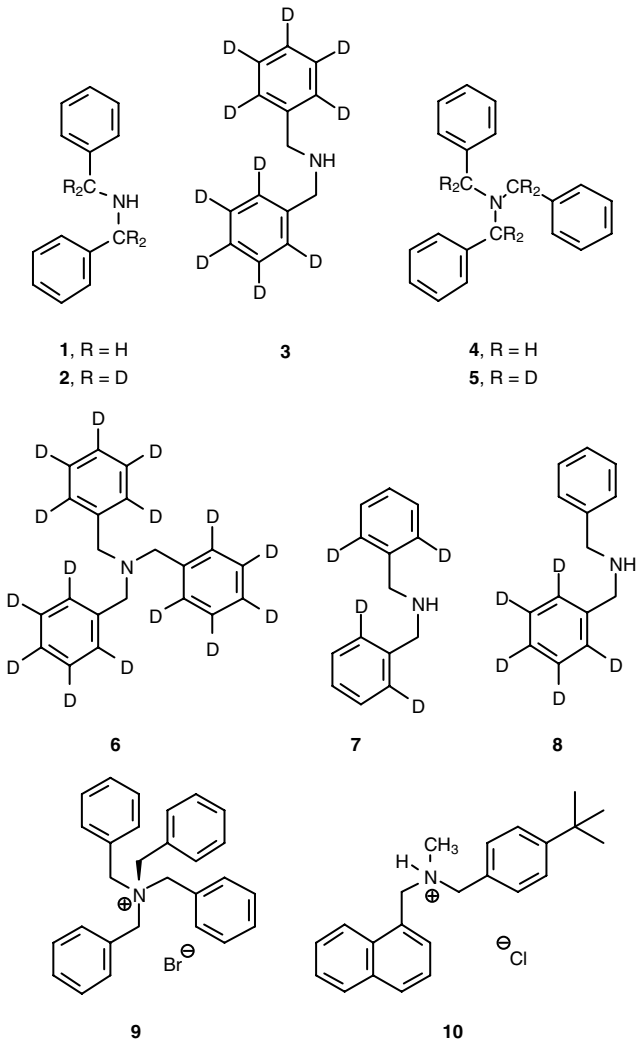

Figure 1. Compounds used for fragmentation studies. Dibenzylamine (**1**), *N*,*N*-bis([1′,1′-$^2H_2$]benzyl)amine (**2**), *N*,*N*-bis([2,3,4,5,6-$^2H_5$]benzyl)amine (**3**), tribenzylamine (**4**), *N*,*N*,*N*-tris([1′,1′-$^2H_2$]benzyl)amine (**5**), *N*,*N*,*N*-tris([2,3,4,5,6-$^2H_5$]benzyl)amine (**6**), *N*,*N*-bis([2,6-$^2H_2$]benzyl)amine (**7**), *N*,*N*-([2,3,4,5,6-$^2H_2$]benzyl)benzylamine (**8**), tetrabenzylammonium bromide (**9**), butenafine hydrochloride (**10**).

deuteriated solvents D_2O (99.8 atom% D; Aldrich Chemical Co., St Louis, MO), and CD_3COOD (99 atom% D; Cambridge Isotope Lab., MA, USA) were used.

General procedures

All solvents and reagents were obtained from commercial suppliers (Aldrich Chemical Co., St Louis, MO) and used as purchased. Butenafine HCl was isolated from the anti-fungal cream Lotrimin Ultra® (Schering-Plough HealthCare Products, Berkeley Heights, NJ). All synthetic products were detected as one spot on thin-layer chromatography analysis (precoated 0.25 mm silica gel plates purchased from Fisher Scientific, Hampton, NH) unless specified. All NMR spectra were recorded from a Varian INOVA 400, a GE 300 or a Varian VXR-200 spectrometer using $CDCl_3$ as solvent. Chemical shifts for ^{1}H NMR are reported in ppm relative to the internal reference TMS. All coupling constants (*J* values) are reported in Hertz (Hz). All ^{13}C NMR spectra were decoupled and all chemical shifts are reported relative to that of $CDCl_3$. EI Mass spectra were recorded on an HP 5970 series Mass Selective Detector coupled to a Hewlett Packard 5890 Series II gas chromatograph. Accurate mass determinations were carried out on a Waters Micromass Q-Tof API-US mass spectrometer equipped with a nanospray source, at a resolution of about 15 000.

Chemical synthesis

Benzyl alcohol

Benzoic acid (0.150 g, 1.23 mmol) in THF (0.5 ml) was added to $LiAlH_4$ (0.072 g, 1.90 mmol) in diethyl ether (0.5 ml). The reaction mixture was stirred at ambient temperature for 2 h and quenched with water (0.08 ml). The inorganic solids were filtered and washed with diethyl ether. The combined ether filtrate was dried over Na_2SO_4 and evaporated to give a colorless oil (0.097 g, 72%). ^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.33 (5H, m), 4.61 (2H, s), 1.94 (1H, s); ^{13}C NMR: (300 MHz, $CDCl_3$) δ 140.81, 128.39, 127.44, 126.87, 64.99; EI-MS: *m*/*z* (%), 108 ($M^{+\bullet}$, 80), 107 (65), 91 (20), 79 (100), 77 (70), 65 (10), 51 (30), 39 (20).

Benzyl bromide

Benzyl alcohol (0.097 g, 0.90 mmol) in acetonitrile (5 ml) was brominated with triphenylphosphine (0.378 g, 1.45 mmol) and bromine (0.24 g, 1.5 mmol).[6] The reaction mixture was heated for 2 h at 55 °C and worked up with 30% hydrogen peroxide (0.5 ml) in 5% aqueous $NaHCO_3$ (10 ml). The aqueous mixture was extracted with pentane–diethyl ether (2 : 1). The organic layer was separated, dried over Na_2SO_4, concentrated, and chromatographed on silica gel (pentane–ether 2 : 1). The eluate was evaporated to give benzyl bromide (0.12 g, 80% yield). ^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.36 (5H, m), 4.48 (2H, s); ^{13}C NMR: (300 MHz, $CDCl_3$) δ 144.03, 128.99, 128.78, 128.40, 46.43; EI-MS: *m*/*z* (%), 170/172 ($M^{+\bullet}$, 10/10), 91 (100), 65 (20), 39 (15).

*Dibenzylamine **(1)***

Benzyl bromide (0.010 g, 0.058 mmol) was added to a stirred solution of acetonitrile (1 ml) and tetrahydrofuran (0.5 ml) and 30% aqueous ammonia (0.125 ml, 2.20 mmol). The reaction mixture was stirred for 1 h at 60 °C, diluted with water and extracted with diethyl ether. The organic layer was separated, concentrated, applied on a preparative thin-layer chromatography plate and eluted with hexanes–ethyl acetate (4 : 1). The product was isolated as a colorless oil from the second major band (R_f = 0.45)(0.003 g, 25%). ^{1}H NMR: (400 MHz, $CDCl_3$) δ 7.34 (8H, m), 7.27 (2H, m), 3.82 (4H, s), 1.76 (1H, s); ^{13}C NMR: (400 MHz, $CDCl_3$) δ 140.28, 128.39, 128.15, 126.95, 53.13; EI-MS: *m*/*z* (%), 197 ($M^{+\bullet}$, 10), 196 (15), 120 (10), 106 (60), 91 (100), 65 (20), 39 (10); Accurate mass ESI-MS $[M + H]^+$ for $C_{14}H_{16}N$ calc. 198.1286, found 198.1283.

*Tribenzylamine **(4)***

Benzyl bromide (0.010 g, 0.058 mmol) was added to a stirred solution of acetonitrile (1 ml) and tetrahydrofuran (0.5 ml) and 30% aqueous ammonia (0.025 ml, 0.44 mmol). The

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reaction mixture was stirred for 1 h at 60 °C, diluted with water, and extracted with diethyl ether. The organic layer was separated, concentrated and applied to a preparative thin-layer chromatography plate. The plate was eluted with hexanes–ethyl acetate (10 : 1). The product was isolated as a white solid from the major band ($R_f = 0.85$) (0.003 g, 25%). ^{1}H NMR: (400 MHz, $CDCl_3$) δ 7.41 (6H, d, $J = 7.2$ Hz), 7.31 (6H, t, $J = 7.6$ Hz), 7.24 (3H, t, $J = 6.8$ Hz), 3.56 (6H, s); ^{13}C NMR: (400 MHz, $CDCl_3$) δ 139.64, 128.73, 128.20, 126.83, 57.91; EI-MS: m/z (%), 287 ($M^{+\bullet}$, 20), 210 (20), 196 (20), 91 (100), 65 (20), 39 (10); Accurate mass ESI-MS $[M + H]^+$ for $C_{21}H_{22}N$ calc. 288.1752, found 288.1757.

[1′,1′-$^2H_2$]Benzyl alcohol

Benzoic acid (0.150 g, 1.21 mmol) in THF (0.5 ml) was reduced by $LiAlD_4$ (0.072 g, 1.71 mmol) in diethyl ether (0.5 ml) by a procedure similar to that described for benzyl alcohol. The product was obtained as a colorless oil (0.097 g, 72%). ^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.34 (5H, m), 1.93 (1H, s); ^{13}C NMR: (300 MHz, $CDCl_3$) δ 140.82, 128.39, 127.44, 126.85, 65.01; EI-MS: m/z (%), 110 ($M^{+\bullet}$, 80), 109 (60), 92 (20), 81 (80), 79 (60), 67 (10), 52 (20), 40 (10).

[1′,1′-$^2H_2$]Benzyl bromide

[1′,1′-$^2H_2$]Benzyl alcohol (0.097 g, 0.88 mmol) in acetonitrile (5 ml) was brominated with triphenylphosphine (0.378 g, 1.45 mmol) and bromine (0.24 g, 1.5 mmol) by a procedure similar to that described for benzyl bromide to give [1′,1′-$^2H_2$]benzyl bromide (0.12 g, 80% yield). ^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.37 (5H, m); ^{13}C NMR: (300 MHz, $CDCl_3$) δ 144.09, 129.00, 128.77, 128.42, 46.44; EI-MS: m/z (%), 172/174 ($M^{+\bullet}$, 10/10), 93 (100), 66 (15), 40 (10).

N,N-*Bis[1′,1′-$^2H_2$]benzylamine* ***(2)***

[1′,1′-$^2H_2$]Benzyl bromide (0.010 g, 0.058 mmol) was added to a stirred solution of acetonitrile (1 ml) and tetrahydrofuran (0.5 ml) and 30% aqueous ammonia (0.125 ml, 2.20 mmol). The reaction mixture was stirred for 1 h at 60 °C, diluted with water and extracted with diethyl ether. The organic layer was separated, concentrated and applied to a preparative thin-layer chromatography plate. The plate was eluted with hexanes–ethyl acetate (4 : 1). The product was isolated as a colorless oil from the second major band ($R_f = 0.45$)(0.003 g, 25%). ^{1}H NMR: (400 MHz, $CDCl_3$) δ 7.34 (8H, m), 7.27 (2H, m), 1.70 (1H, s); ^{13}C NMR: (200 MHz, $CDCl_3$) δ 140.28, 128.40, 128.18, 126.98, 53.14; ^{1}H NMR: (400 MHz, $CDCl_3$); EI-MS: m/z (%), 201 ($M^{+\bullet}$, 10), 200 (5), 124 (10), 108 (40), 93 (100), 67 (10), 40 (10); Accurate mass ESI-MS $[M + H]^+$ for $C_{14}H_{12}{}^2H_4N$ calc. 202.1534, found 202.1534.

N,N,N-*Tris[1′,1′-$^2H_2$]benzylamine* ***(5)***

[1′,1′-$^2H_2$]Benzyl bromide (0.010 g, 0.058 mmol) was added to a stirred solution of acetonitrile (1 ml) and tetrahydrofuran (0.5 ml) and 30% aqueous ammonia (0.025 ml, 0.44 mmol). The reaction mixture was stirred for 1 h at 60 °C, diluted with water and extracted with diethyl ether. The organic layer was separated, concentrated and applied to a preparative thin-layer chromatography plate. The plate was eluted with hexanes–ethyl acetate (10 : 1). The product was obtained as a white solid from the major band ($R_f = 0.85$) (0.003 g, 25%). ^{1}H NMR: (400 MHz, $CDCl_3$) δ 7.40 (6H, td, $J = 8.4$, 1.2 Hz), 7.31 (6H, tt, $J = 7.6$, 1.6 Hz), 7.22 (3H, tt, $J = 7.2$, 1.6 Hz); ^{13}C NMR: (400 MHz, $CDCl_3$) δ 139.54, 128.76, 128.20, 126.84, 57.92; EI-MS: m/z (%), 293 ($M^{+\bullet}$, 20), 216 (25), 200 (20), 93 (100), 67 (10), 40 (5); Accurate mass ESI-MS $[M + H]^+$ for $C_{21}H_{16}{}^2H_6N$ calc. 294.2129, found 294.2133.

[2,3,4,5,6-$^2H_5$]Benzyl alcohol

[2,3,4,5,6-$^2H_5$]Benzoic acid (0.150 g, 1.18 mmol) in THF (0.5 ml) was reduced with $LiAlD_4$ (0.072 g, 1.71 mmol) in diethyl ether (0.5 ml) by a procedure similar to that described for benzyl alcohol to give a colorless oil (0.097 g, 72%). ^{1}H NMR: (300 MHz, $CDCl_3$) δ 4.62 (2H, s), 1.92 (1H, s); ^{13}C NMR: (300 MHz, $CDCl_3$) δ 140.83, 128.41, 127.47, 126.86, 64.97; EI-MS: m/z (%), 113 ($M^{+\bullet}$, 100), 112 (40), 96 (20), 83 (90), 81 (40), 68 (10), 54 (40), 42 (15).

[2,3,4,5,6-$^2H_5$]Benzyl bromide

[2,3,4,5,6-$^2H_5$]Benzyl alcohol (0.097 g, 0.86 mmol) in acetonitrile (5 ml) was brominated with triphenylphosphine (0.378 g, 1.45 mmol) and bromine (0.24 g 1.5 mmol) by a procedure similar to that described for benzyl bromide to give the desired product (0.12 g, 80% yield). ^{1}H NMR: (300 MHz, $CDCl_3$) δ 4.49 (2H, s); ^{13}C NMR: (300 MHz, $CDCl_3$) δ 144.01, 128.98, 128.78, 128.40, 46.45; EI-MS: m/z (%), 175/177 ($M^{+\bullet}$, 10/10), 96 (100), 69 (15), 42 (10).

N,N-*Bis[2,3,4,5,6-$^2H_5$]benzylamine* ***(3)***

[2,3,4,5,6-$^2H_5$]Benzyl bromide (0.010 g, 0.057 mmol) was added to a stirred solution of acetonitrile (1 ml) and tetrahydrofuran (0.5 ml) and 30% aqueous ammonia (0.125 ml, 2.20 mmol). The reaction mixture was stirred for 1 h at 60 °C, diluted with water, extracted with diethyl ether, and the product was isolated by preparative thin-layer chromatography (hexanes-ethyl acetate, 4 : 1) as a colorless oil was from the second major band at $R_f = 0.45$ (0.003 g, 25%). ^{1}H NMR: (400 MHz, $CDCl_3$) δ 3.82 (4H, s), 1.69 (1H, s); ^{13}C NMR: (400 MHz, $CDCl_3$) δ 140.29, 128.38, 128.15, 126.94, 53.14; EI-MS: m/z (%), 207 ($M^{+\bullet}$, 10), 206 (15), 125 (10), 111 (50), 96 (100), 68 (15), 42 (5); Accurate mass ESI-MS $[M + H]^+$ for $C_{14}H_6{}^2H_{10}N$ calc. 208.1910, found 208.1914.

N,N,N-*Tris[2,3,4,5,6-$^2H_5$]benzylamine* ***(6)***

[2,3,4,5,6-$^2H_5$]Benzyl bromide (0.010 g, 0.057 mmol) was added to a stirred solution of acetonitrile (1 ml) and tetrahydrofuran (0.5 ml) and 30% aqueous ammonia (0.025 ml, 0.44 mmol). By a procedure similar to that used for *N,N,N*-tris[1′,1′-$^2H_2$]benzylamine (**5**), a white solid was obtained from the major TLC band ($R_f = 0.85$) (0.003 g, 25%). ^{1}H NMR: (400 MHz, $CDCl_3$) δ 3.58 (6H, s); ^{13}C NMR: (400 MHz, $CDCl_3$) δ 139.66, 128.74, 128.20, 126.84, 57.90; EI-MS: m/z (%), 302 ($M^{+\bullet}$, 20), 220 (20), 107 (10), 96 (100), 68 (15), 42 (5); Accurate mass ESI-MS $[M + H]^+$ for $C_{21}H_7{}^2H_{15}N$ calc. 303.2694, found 303.2700.

[2,6-$^2H_2$]Toluene

2,6-Dibromotoluene (0.040 g, 0.16 mmol) and $LiAlD_4$ (0.030 g) were mixed with diethyl ether (0.30 ml) and heated

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overnight in a sealed tube at 80 °C. The tube was cooled to RT and several drops of water were added to quench excess reducing agent. The product was extracted with pentane and dried over $MgSO_4$. The solvent was removed under reduced pressure at 0 °C to afford a clear liquid (0.010 g, 67%). ^{1}H NMR: (400 MHz, $CDCl_3$) δ 7.25 (2H, d, $J = 7.6$ Hz), 7.16 (1H, t, $J = 7.2$ Hz), 2.35 (3H, s); EI-MS: m/z (%), 94 ($M^{+\bullet}$, 75), 93 (100), 66 (10), 52 (10), 40 (15).

[2,6-$^2H_2$]Benzyl bromide

N-Bromosuccinimide (0.017 g, 0.096 mmol) was added to a solution of [2,6-$^2H_2$]toluene (0.010 g, 0.11 mmol), carbon tetrachloride (0.5 ml) and benzoyl peroxide (1 mg) The mixture was heated in a sealed vial at 80 °C for 4 h. Pentane (2 ml) was added and the organic layer was washed with water (2 × 2 ml), and dried over $MgSO_4$. The solvent was removed under reduced pressure at 0 °C to afford crude [2,6-$^2H_2$]benzyl bromide (0.010 g, 60%). EI-MS: m/z (%), 172/174 ($M^{+\bullet}$, 10/10) 93 (100), 66 (15), 40 (10).

N,N-*Bis[2,6-$^2H_2$]benzylamine* ***(7)***

N-Bromosuccinimide (0.017 g, 0.96 mmol) was added to a solution of [2,6-$^2H_2$]toluene (0.010 g, 0.11 mmol), chloroform (0.5 ml), and benzoyl peroxide (1 mg). The mixture was heated in a sealed vial at 80 °C for 4 h. Acetonitrile (2 ml) was added to the mixture followed by 30% aqueous ammonia (0.250 ml, 4.41 mmol). The mixture was heated at 60 °C for 2 h and the solvents were removed under reduced pressure. Two drops of 12 M hydrochloric acid was added to the residue to form a salt. The acid was evaporated under reduced pressure and the residue was washed with ether to yield a pure white solid (0.003 g, 35%). A small portion of the hydrochloride salt was converted to the free amine for EI-MS analysis. ^{1}H NMR: (400 MHz, $CDCl_3$) δ 7.45 (2H, t, $J = 7.6$ Hz), 7.26 (1H, d, $J = 7.2$ Hz), 4.15 (6H, s) 1.62 (1H, s); EI-MS: m/z (%), 201 ($M^{+\bullet}$, 10), 200 (15), 122 (10), 108 (50), 93 (100), 66 (15), 40 (10); Accurate mass ESI-MS $[M + H]^+$ for $C_{14}H_{12}{}^2H_4N$ calc. 202.1528, found 202.1526.

N-*[2,3,4,5,6-$^2H_5$]Benzyl*-N-*benzylamine* ***(8)***

[2,3,4,5,6-$^2H_5$]Benzyl bromide (0.010 g, 0.06 mmol) in diethyl ether (0.10 ml) was added to a stirred solution of benzylamine (0.014 g, 0.13 mmol) in THF (0.20 ml). The reaction mixture was heated at 60 °C for 2 h, washed with 5% aqueous $NaHCO_3$ (2 × 2 ml) and evaporated to give a mixture of the product *N*-[2,3,4,5,6-$^2H_5$]benzyl-*N*-benzylamine and benzylamine. EI-MS: m/z (%), 202 ($M^{+\bullet}$, 25), 201 (20), 125 (10), 120 (10), 111 (55), 106 (50), 96 (100), 91 (100); Accurate mass ESI-MS $[M + H]^+$ for $C_{14}H_{11}{}^2H_5N$ calc. 203.1591, found 203.1593.

Tetrabenzyl ammonium bromide ***(9)***

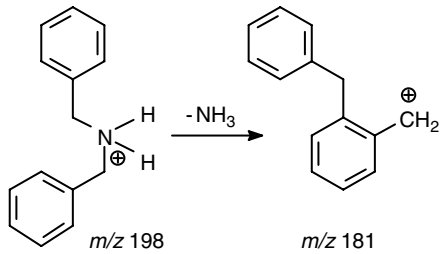
Tribenzylamine (0.010 g, 0.035 mmol) in dry THF (0.2 ml) was added to a stirred solution of benzyl bromide (0.010 g, 0.06 mmol) in dry diethyl ether (0.1 ml). The reaction mixture was heated at 60 °C for 2 h. The white precipitate formed was filtered and washed with ether to yield a mixture (0.017 g) of tribenzylamine hydrobromide and the product tetrabenzylammonium bromide. Accurate mass ESI-MS $[M - Br]^+$ for $C_{28}H_{28}N$ calc. 378.2216, found 378.2220.

RESULTS AND DISCUSSION

The CID spectrum of protonated dibenzylamine (**1,** Fig. 1) shows a significant peak at m/z 181 for a loss of NH_3 (Fig. 2(A)). Although such a loss appears trivial, the mechanism is not straightforward since the protonated molecule should undergo a complicated skeletal rearrangement to eliminate a molecule of ammonia.

For example, to eliminate a molecule of ammonia from protonated dibenzylamine, two carbon–nitrogen bonds must be broken, the charge on the nitrogen should be neutralized, the two benzyl groups must be connected to form the m/z 181 ion, and a hydrogen must be transferred to the nitrogen atom (Scheme 1). Interestingly, the CID spectrum of protonated tribenzylamine (**4**) (Fig. 3(A)) also shows a peak at m/z 181 for a corresponding neutral loss of benzylamine. Accurate mass determinations showed that the m/z 181 peak represents $C_{14}H_{13}{}^+$ (Table 1). We undertook extensive studies of isotope-labeled compounds to ascertain the mechanisms underlying the formation of the m/z 181 and other product ions of protonated benzylamines. The compounds employed in the investigation are depicted in Fig. 1.

Interestingly, deuterium exchange experiments of dibenzylamine (**1**) and tribenzylamine (**4**) ascertained that the exchangeable hydrogens remained bonded to nitrogen during CID fragmentation to eliminate ammonia (Figs 2(B) and 3(B)). The seemingly palpable source for the additional hydrogen required for the elimination of ammonia from protonated dibenzylamine is the adjacent benzylic hydrogens. However, when the benzylic hydrogens were replaced



Scheme 1. Elimination of ammonia from protonated dibenzylamine.

Table 1. Accurate masses of product ions derived from protonated dibenzylamine (**1**) and tribenzylamine (**4**)

Compound	Nominal m/z	Elemental composition	Accurate mass (calculated)	Observed mass[a]	Relative error (ppm)
1	181	$C_{14}H_{13}{}^+$	181.1017	181.1018	0.4
	106	$C_7H_8N^+$	106.0657	106.0655	−1.5
	91	$C_7H_7{}^+$	91.0548	91.0547	−0.7
4	196	$C_{14}H_{14}N^+$	196.1126	196.1126	0.1
	181	$C_{14}H_{13}{}^+$	181.1017	181.1020	1.5
	120	$C_8H_{10}N^+$	120.0813	120.0815	1.6
	91	$C_7H_7{}^+$	91.0548	91.0547	−1.0

[a] Accurate masses were determined on the Q-Tof instrument at $R \approx 15\,000$.

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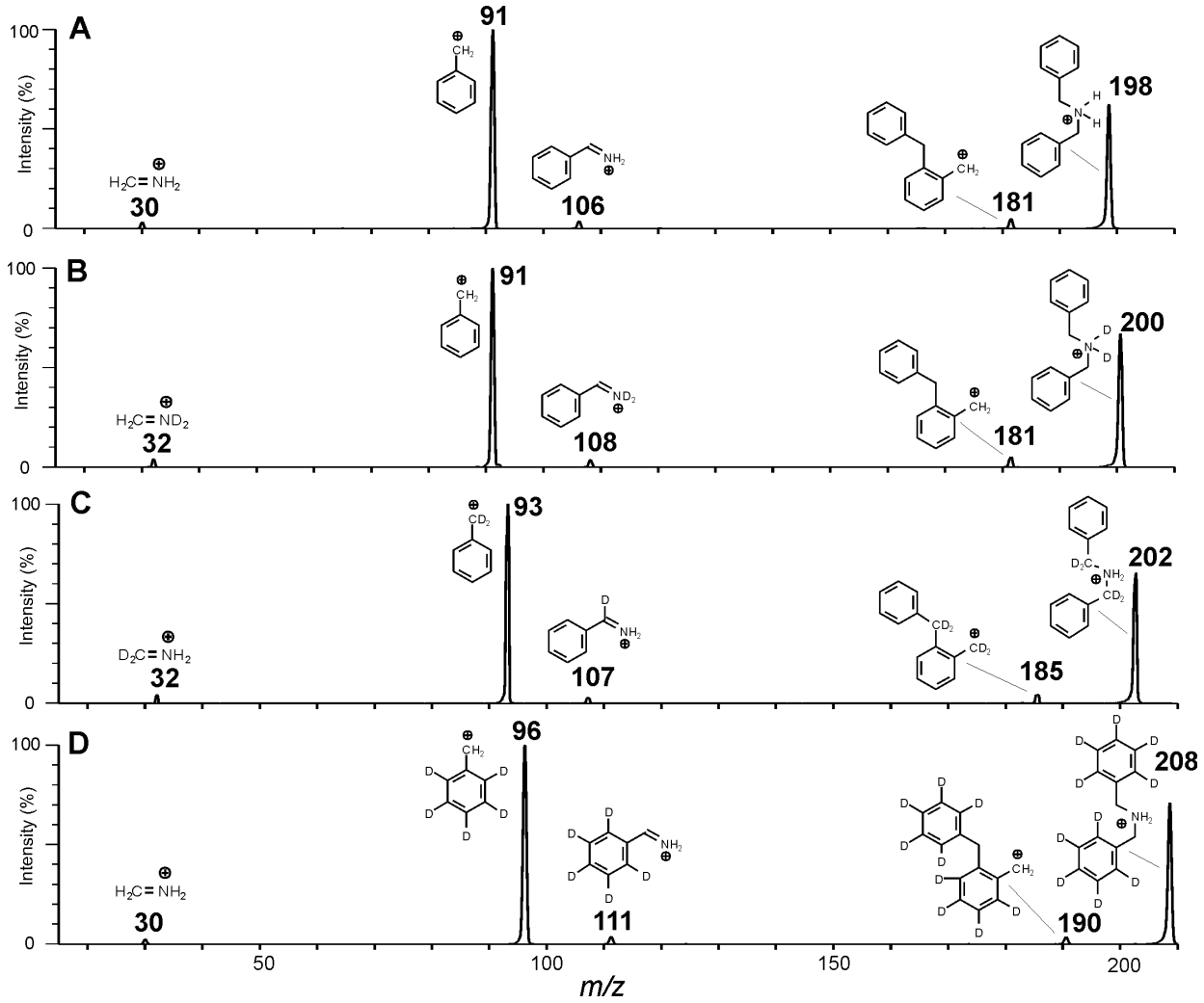
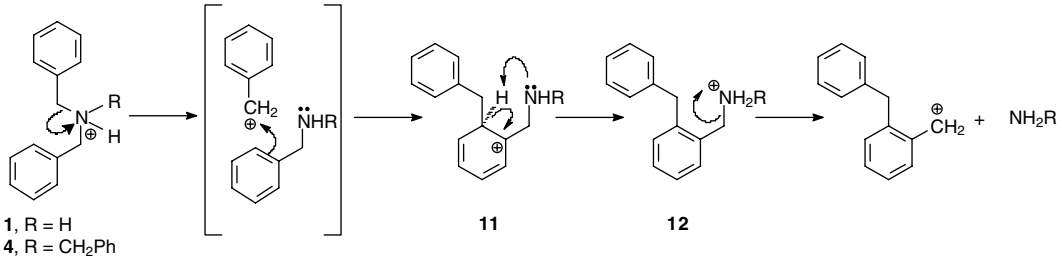


Figure 2. Product ion spectra of $[M + H]^+$ ions of *m/z* 198 of dibenzylamine (**1**) (A), *m/z* 200 of dibezylamine recorded in D_2O (B), *m/z* 202 of *N*, *N*-bis([1′,1′-$^2H_2$]benzyl)amine (**2**) (C), and *m/z* 208 of *N*, *N*-bis([2,3,4,5,6-$^2H_5$]benzyl)amine (**3**) (D), recorded at a collision energy setting of 15 eV.

by deuterons and the spectrum of the deuteriated benzylamine (**2**) was recorded, to our surprise no deuterium atom was eliminated in the process of eliminating an ammonia molecule (Fig. 2(C)). Similarly, benzylic hydrogens do not provide the extra hydrogen atom required for the elimination of a benzylamine molecule from protonated tribenzylamine (Fig. 3(C)). It follows that a hydrogen from a phenyl ring must transfer specifically to the nitrogen atom during the elimination of ammonia or benzylamine. Support for this proposition was obtained from the CID spectra of ring-deuteriated amines **3** and **6**. As expected, a deuterium atom from a phenyl ring was exclusively eliminated in the process of budding an ammonia (Fig. 2(D), peak at *m/z* 190) or a benzylamine molecule (Fig. 3(D), peak at *m/z* 190).

In order to rationalize these observations, we postulate the mechanism given in Scheme 2. An initial elongation of a C–N bond at the charge center leads to the consequent cleavage of the C–N bond forming an ion/neutral complex of benzyl cation and benzylamine. A simple dissociation of the complex leads to the benzyl cation that represents the most intense peak observed at *m/z* 91, or 93 and 96 for deuterated species, in Figs 2 and 3. On the other hand, the



Scheme 2. Fragmentation mechanism proposed for elimination of ammonia or benzylamine from protonated benzylamines.

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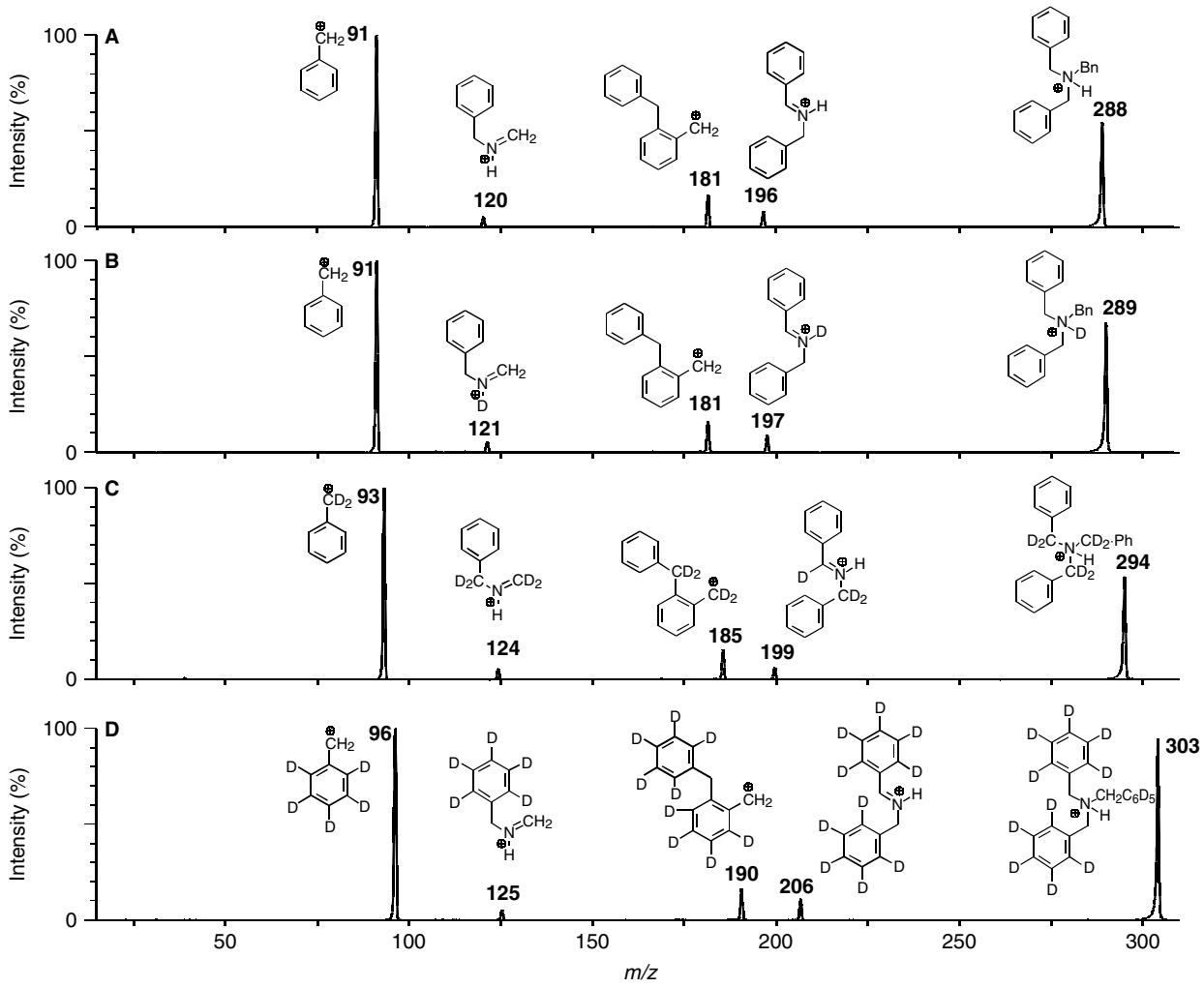
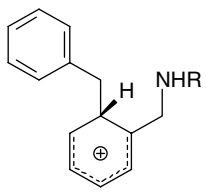


Figure 3. Product ion spectra of $[M + H]^+$ of *m/z* 288 of tribenzylamine (**4**) (A), *m/z* 289 of tribenzylamine recorded in D_2O (B), *m/z* 294 of *N*,*N*,*N*-tris([1′,1′-$^2H_2$]benzyl)amine (**5**) (C), and *m/z* 303 of *N*,*N*,*N*-tris([2,3,4,5,6-$^2H_5$]benzyl)amine (**6**) (D), recorded at a collision energy setting of 20 eV.

benzyl cation could initiate an electrophilic attack on the *ipso*, *ortho*, *meta*, or *para* positions of the benzylamine by removing a pair of electrons from the aromatic sextet yielding an arenium ion (**11**, Scheme 2).[7] Although the arenium ion bears less stability than that associated with aromatic systems, the ion carries some stability of its own by resonance. A more realistic structure for this highly reactive arenium ion is that illustrated as structure **13**.



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The highly reactive arenium ion then stabilizes itself by regenerating the aromatic ring by donating a ring proton (or deuteron when present) to the amino group, thereby generating a protonated amine intermediate **12** (Scheme 2), which could undergo a charge-driven heterolytic cleavage[8] to eliminate ammonia (from amine **1**; R═H), or benzylamine (from amine **4**; R═CH_2Ph), thus forming a benzylbenzyl cation[9,10] that is observed at *m/z* 181 (Figs 2 and 3). The benzylbenzyl cation could well undergo a ring closure to yield a protonated dihydroanthracene isomer as described by Grützmacher and Dohmeier-Fischer.[11] Moreover, the mechanism proposed for the loss of ammonia observed in the CID spectrum of protonated noradrenaline, a related compound, could well undergo a transition through an arenium ion.[12] It is well known that intramolecular proton exchanges occur in arenium ions at a very rapid phase. For example, 11 ring hydrogens in protonated α,ω-diphenylalkanes are known to randomize within a span of 1 μs (Scheme 3).[13] Protons are deemed to 'ring walk' in isolated arenium ions via an edge-protonated species.[14] If this model is valid, the hydrogen required for the elimination of an ammonia molecule should not originate specifically from the *ortho* position.

In fact, the CID spectrum of protonated compound **7** (dibenzylamine with *ortho*-dideuteriated phenyl rings) supports the 'proton dance' model. The spectrum shows peaks for the loss of both NH_3 and NDH_2 at *m/z* 185, and 184, respectively, at an approximate ratio of 3 to 2 (Fig. 4(A)). On

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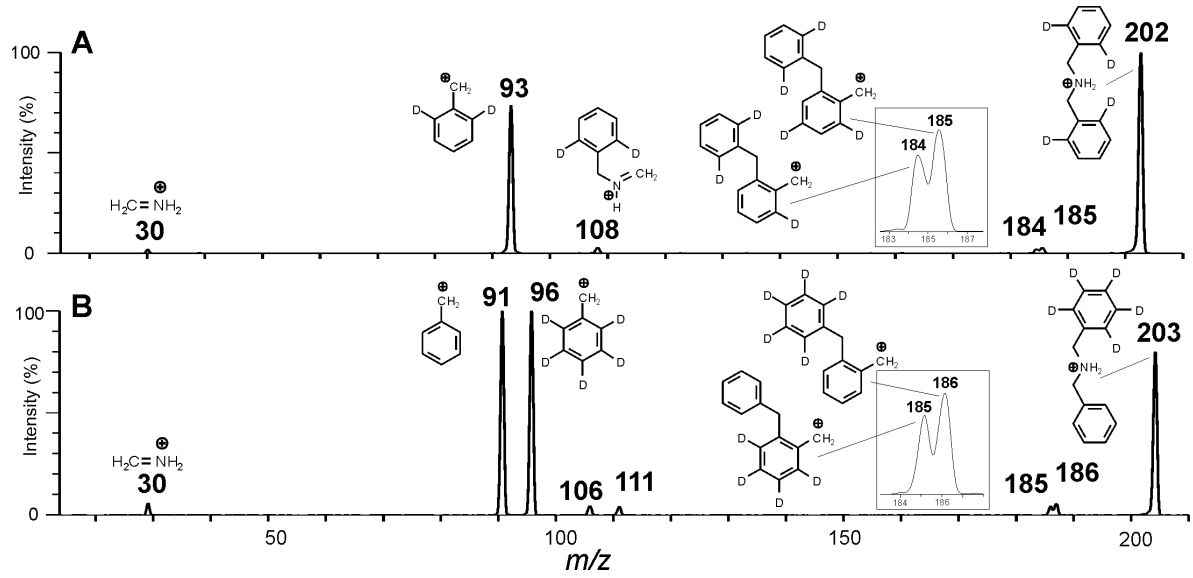
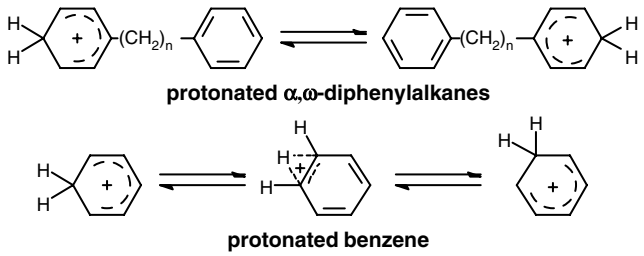


Figure 4. Product ion spectra of $[M + H]^+$ of m/z 202 of *N*, *N*-bis([2,6-$^2H_2$]benzyl)amine (**7**) (A), and m/z 203 of *N*, *N*-([2,3,4,5,6-$^2H_2$]benzyl)benzylamine (**8**) (B), recorded at a collision energy setting of 15 eV. (Insets show expansion of m/z 183–187 region).

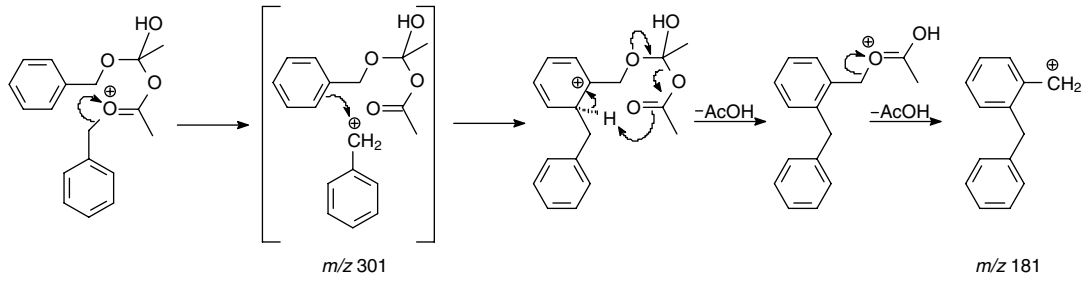


Scheme 3. Ring Walk mechanism in arenium ions proposed by Kuck *et al*. (Refs 13 and 14).

the other hand, the CID spectrum of protonated compound **8** (dibenzylamine with one perdeuterated benzene ring), which demonstrates a favored loss of NH_3 over that of NDH_2 even though both rings are statistically equivalent, provided confirmative evidence for the favored breakage of a C–H bond over a C–D bond owing to an isotope effect (Fig. 4(B)). In the presence of such a pronounced isotope effect, it is clear that a loss of NDH_2 is somewhat biased over that of NH_3 (original *ortho* protons are eliminated more rapidly than the others). The most plausible explanation for the observed ratios is that the complete H/D equilibrium had not been achieved before the rearomatization took place by donating a proton to the amine group.

The formation of a benzylbenzyl cation (m/z 181) in gas phase has been well investigated.[14] For example, the reaction of gaseous benzyl cations with benzyl methyl ether leads to m/z 181 as a condensation product.[11] Similarly, the fragmentation of a protonated dimer formed under chemical ionization of benzyl acetate produces m/z 181.[10] The formation of this m/z 181 ion from the protonated dimer of benzyl acetate had been rationalized by two consecutive losses of acetic acid. However, no attempts had been made to confirm the exact origin of the transferred hydrogen atoms during the rearrangement. Applying the arenium ion mechanism discussed here, we propose a rationale for the consecutive acetic acid losses, in which an aromatic proton is eliminated during the loss of the first acetic acid molecule (Scheme 4).

A comparison of CID spectra of protonated dibenzylamine (**1**) and tribenzylamine (**4**) revealed another interesting feature. Under identical CID conditions, protonated compound **4** produces a more intense signal at m/z 181 than compound **1**. We asked ourselves if the tetrabenzylammonium ion (**9**) would generate an even more intense m/z 181 signal than that of tribenzylammonium ion under identical conditions. As expected, the CID spectrum of the tetrabenzylammonium ion showed a significantly more intense signal at m/z 181 than those of protonated amines **1** and **4**



Scheme 4. Mechanism proposed for fragmentation of protonated dimer of benzyl acetate.

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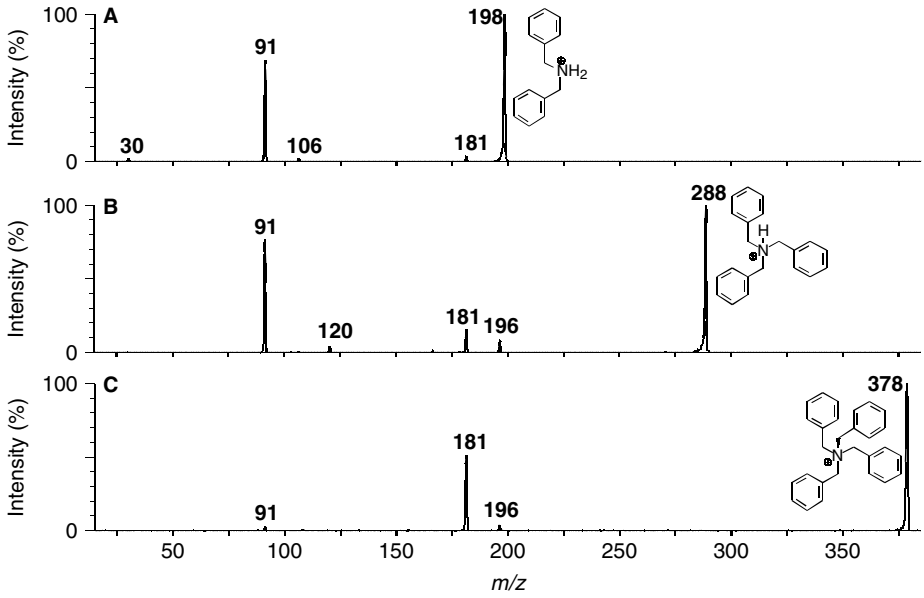
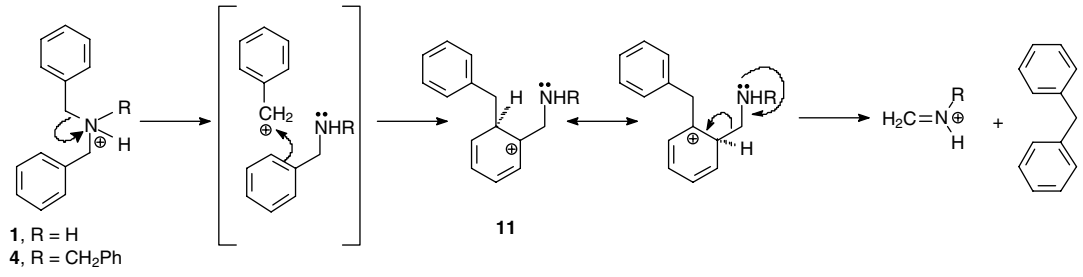


Figure 5. Product ion spectra of $[M + H]^+$ of *m/z* 198 dibenzylamine (**1**) (A), *m/z* 288 of tribenzylamine (**4**) (B), and that of *m/z* 378 of $[M - Br]^+$ of tetrabenzylammonium bromide (**9**) (C). All spectra were recorded under the same conditions and settings (collision energy 18 eV) over a period of 1 h.

(Fig. 5). Moreover, the tetrabenzylammonium ion produced a drastically low-intense benzyl-cation signal at *m/z* 91. Presumably, the formation of the arenium ion leading to *m/z* 181 ion relieves the steric congestion of the ion/neutral complex more effectively than that delivered by a direct formation of the benzyl cation and tribenzylamine. To a lesser extent, steric effects must also play a role in the fragmentation of protonated tribenzylamine (**4**) as well. However, the electrophilic attack of the benzyl cation on the amine is most favorable with the ion/neutral complex derived from the tetrabenzylammonium ion (**9**). This is due to the fact that the benzyl groups are more numerous and more closely located in the ion/neutral complex of tetrabenzylammonium ion than those derived from protonated tribenzylamine (**4**) and dibenzylamine (**1**). Consequently, the formation of *m/z* 181 would then be expected to be less favorable in the case of **4,** and even lesser in **1**, which in fact is congruent with the observed data.

Beside *m/z* 181, CID spectra of protonated amines **1** and **4** show a pair of low-intensity signals at *m/z* 30 and 106, and 120 and 196, respectively (Figs 2(A) and 3(A)). We postulate that the ions that correspond to peaks at *m/z* 30 and *m/z* 120 in the spectra of protonated amines **1** and **4** originate by eliminating a molecule of diphenylmethane (168 Da). Since both product ions are of even mass and contain one nitrogen atom, the probable structure for the *m/z* 30 ion must be $H_2C{=}NH_2^+$, while that for the *m/z* 120 ion should be $H_2C{=}NH^+CH_2Ph$. The proposed formula for the benzylimmonium ion is supported by its accurate mass (Table 1). The proposed loss of diphenylmethane is interesting because it shows similarities to the formation of *m/z* 181. In order to eliminate a diphenylmethane molecule, a carbon–nitrogen bond and carbon–carbon bond must be broken and the two aromatic groups must be reconnected. We asked ourselves if the benzyl groups are connected through a similar mechanism involving the formation of the arenium ion. The CID spectra of protonated deuterated dibenzylamine (**2**) and tribenzylamine **(5)** showed a loss of $[^2H_2]$diphenylmethane (170 Da) to give peaks at *m/z* 32 and *m/z* 124 (Figs 2(C) and 3(C)), respectively, indicating only one methylene group gets eliminated with the leaving $=NH_2$, or =NHR, moiety. For example, the intermediate arenium ion **11** originating from an *ortho* attack undergoes a charge-driven heterolytic cleavage to restore the aromatic ring, thereby producing the relevant immonium ion (Scheme 5). A similar mechanism can be postulated for the fragmentation of the arenium intermediate that originates from an *ipso* attack (Scheme 6). Support for the postulated pathways was



Scheme 5. Proposed mechanism for elimination of diphenylmethane from protonated di- and tri-benzylamine (*ortho* attack).

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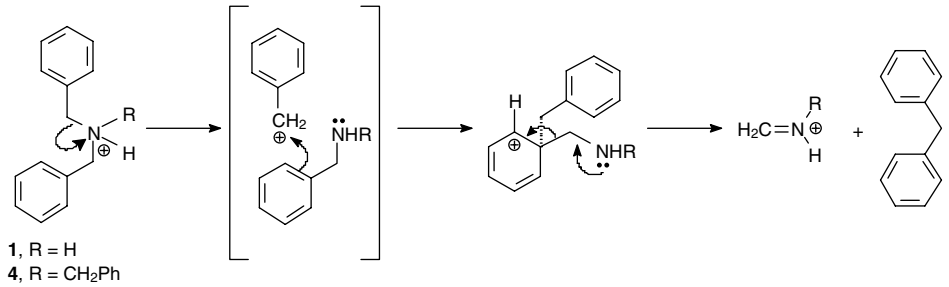
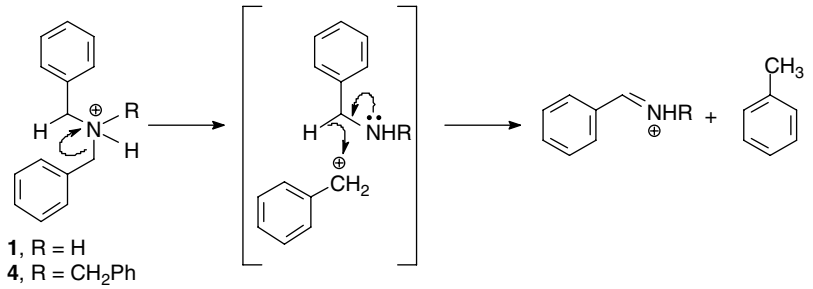


Scheme 6. Proposed mechanism for elimination of diphenylmethane from protonated di- and tri-benzylamine (*ipso* attack).



Scheme 7. Mechanism proposed for elimination of toluene from protonated di- and tri-benzylamine.

obtained by the CID spectra of deuterated compounds **3** and **6**. The elimination of $[^2H_{10}]$diphenylmethane (178 Da) to yield signals at m/z 30 and m/z 125 (Figs 2(D) and 3(D)) indicates that no deuterium exchange occurs from the phenyl rings to the $H_2C{=}NHR^+$ immonium ion during this fragmentation.

Moreover, CID spectra of ions derived from the protonation of amines **1** and **4** show peaks at m/z 106 and m/z 196, respectively, both of which are formed by a significant loss of elements of toluene (Figs 2(A) and 3(A)). Similar to the above-mentioned m/z 30 and m/z 120 ions, the m/z 106 and m/z 196 species are even-mass ions that differ by 90 Da, which formally represents a benzyl group minus one hydrogen. In contrast, CID spectra derived from deuterium-labeled compounds **3** and **6** show a loss of 95 Da for $[^2H_3]$toluene (Figs 2(C) and 3(C)). The accurate masses of m/z 106 and m/z 196 ions agree with proposed elemental compositions (Table 1). This observation indicates that a hydride abstraction from a methylene group of the neutral benzylamine (or dibenzylamine) to the benzylic cation occurs within the proposed ion/neutral complex to eliminate toluene as a neutral fragment (Scheme 7). The CID spectra of deuteriated amines **3** and **6** show peaks at m/z 111 and 206, respectively, for a loss of 97 Da for $[^2H_5]$toluene (Figs 2(D) and 3(D)) corroborating that no deuterium transfer occurs from the ring in this fragmentation. Since the aromatic groups do not join, no arenium ion intermediates are expected. Our mechanism is not unique since similar hydride abstractions have been observed in other ion/neutral complexes that eliminate toluene.[15,16]

The antifungal drug butenafine hydrochloride (**10**) is a salt of a tertiary amine, as well as a modified dibenzylamine. To demonstrate the applicability of our mechanisms, we predicted that compound **10** will undergo CID fragmentations similar to those observed for dibenzylamine and tribenzylamine. In fact, the CID spectrum of butenafine (**10**) showed four low-intensity signals at m/z 287, 176, 170, and

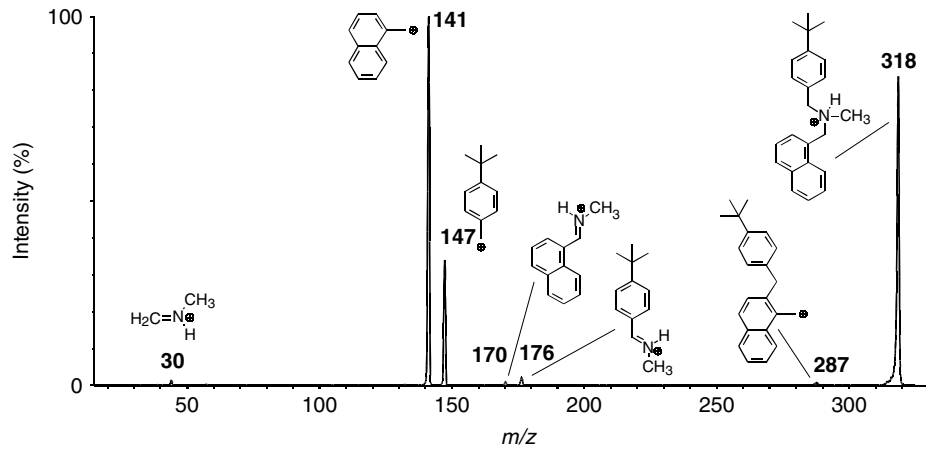


Figure 6. Product ion spectrum of m/z 318 of $[M - Cl]^+$ of butenafine hydrochloride (**10**).

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44, all of which could be accurately predicted by our mechanisms (Fig. 6). This indicates that the fragmentation patterns described are universal, and are useful in predicting CID fragmentations of protonated benzylamine derivatives, several of which are biologically and medicinally important compounds.

Developing predictive rules for low-energy CID spectra is very important for the identification of drugs and their metabolites in biological systems. Using artificial intelligence for this purpose has been attempted, but has not yet been widely successful. Predictive rules formulated without detailed labeling studies could be misleading. Here, we have accomplished a detailed study on the fragmentation of benzylamine derivatives, and have demonstrated how our conclusions can be used for the identification of benzylamine drugs and related compounds.

Acknowledgements

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