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Collision-induced Dissociation Mass Spectra of Cocaine, and its Metabolites and Pyrolysis Products

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As the first step in the development of a liquid chromatographic/tandem mass spectrometric method for the quantitation of cocaine, its metabolites and its pyrolytic degradation products from biological matrices, the collision-induced dissociation of the protonated and selected deuterium-labeled species was studied. The fragment ions generated from the CID mass spectra were assigned and fragmentation mechanisms were proposed. © **1998 John Wiley & Sons, Ltd.**

KEYWORDS: cocaine; collision-induced dissociation; fragmentation mechanisms

INTRODUCTION

Cocaine has a long history of human use and abuse. From a clinical and forensic point of view, the identification and quantitation of cocaine, its metabolites and its pyrolysis products have become very important. Metabolites such as norcocaine are psychoactive whereas other metabolites such as benzoylnorecgonine have been noted to cause seizures. Cocaethylene, norcocaethylene and ecgonine ethyl ester are formed by the transesterification of the methyl ester and are used as diagnostic markers for co-administration of cocaine and ethanol. In addition, when cocaine is smoked two pyrolysis products are formed, anhydroecgonine and anhydroecgonine methyl ester. These pyrolysis products may be used as markers for the route of ingestion of cocaine.

Mass spectrometry, especially gas chromatography/mass spectrometry (GC/MS),[1] has played a pivotal role in the analysis of cocaine for many years owing to its high sensitivity and specificity. Recently, with the advent of electrospray ionization, liquid chromatography/mass spectrometry (LC/MS) has gained in popularity. Compared with GC/MS, LC/MS requires less sample preparation, including no sample derivitization. In addition, some thermally labile metabolites such as cocaine *N*-oxide can be monitored by LC/MS, but not by GC/MS.[2] In this laboratory, we have been developing LC/MS and LC/MS/MS methods to study cocaine and its metabolites. As the first step towards the development of an LC/MS/MS method, a detailed study of the collision-induced dissociation (CID) products of cocaine and its metabolites and pyrolysis products was explored. In this work, we studied the CID mechanism of cocaine and the following related compounds: cocaine *N*-oxide, norcocaethylene, ecgonine methyl ester, benzoylnorecgonine, ecgonine, benzoylecgonine, cocaethylene, norcocaine, *p*-hydroxycocaine, *m*-hydroxycocaine, *p*-hydroxybenzoylecgonine, *m*-hydroxybenzoylecgoine, ecgonine ethyl ester, anhydroecgonine methyl ester and anhydroecgonine. The CID mass spectra of the deuterium-labeled compounds, [*N*-C^2H_3]cocaine (D_3COC), [*N*-C^2H_3]benzoylecgonine (D_3BE) and [*N*-C^2H_3]ecgonine methyl ester (D_3EME) were also studied to confirm the identity of certain fragment ions and to help elucidate the fragmentation mechanisms.

EXPERIMENTAL

Chemicals

(−)-Cocaine hydrochloride (COC), (−)-cocaine *N*-oxide hydrochloride (CNO), (−)-*N*-norcocaethylene fumarate (NCE), (−)-anhydroecgonine methyl ester fumarate (AEME), (−)-ecgonine methyl ester hydrochloride (EME), (−)-benzoylnorecgonine hydrochloride (BN), (−)-ecgonine hydrochloride (ECG), (−)-benzoylecgonine (BE), (−)-cocaethylene fumarate (CE), (−)-*N*-norcocaine (NC), [*N*-C^2H_3]cocaine

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hydrochloride (D_3-COC), [N-C^2H_3]benzoylecgonine (D_3-BE) and [N-C^2H_3] ecgonine methyl ester hydrochloride (D_3-EME) were provided by the National Institute on Drug Abuse (Rockville, MD, USA). *p*-Hydroxycocaine (PHOCOC), *m*-hydroxycocaine (MHOCOC), *p*-hydroxybenzoylecgonine (PHOBE) and *m*-hydroxybenzoylecgonine (MHOBE) were purchased from Research Biochemicals International (Natick, MA, USA). Anhydroecgonine hydrochloride (AECG) and ecgonine ethyl ester (EEE) were obtained from Radian International (Austin, TX, USA). All chemicals were powders except AECG and EEE, which were 1 mg ml^{-1} methanol solutions.

Methanol and acetonitrile (both HPLC grade, J. T. Baker, Philipsburg, NJ, USA), were used without further purification. Deionized water was generated from a Continental (Natick, MA, USA) deionized water system.

Instrumentation

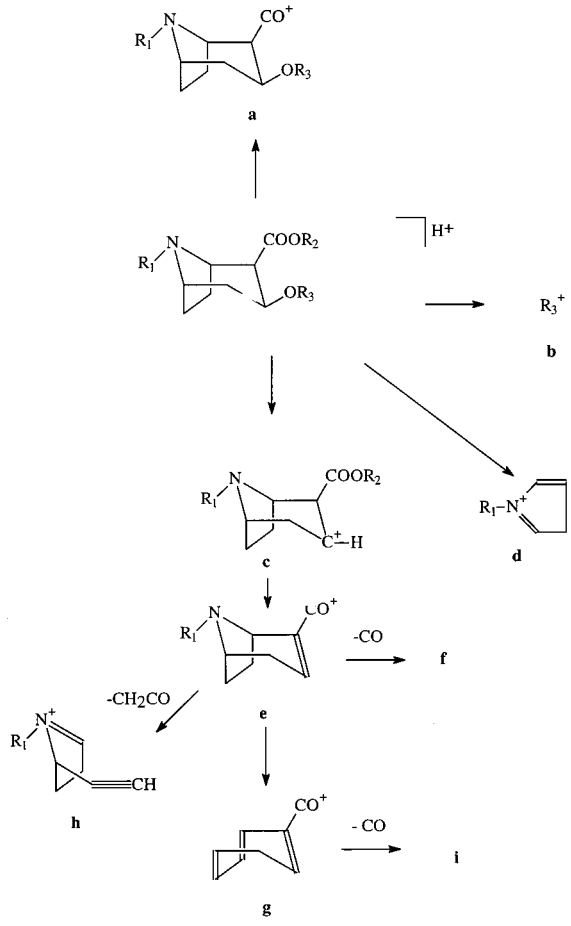
Mass spectrometric experiments were performed using a Micromass (Beverly, MA, USA) Quattro II triple-quadrupole mass spectrometer equipped with an electrospray ionization (ESI) ion source heated to 80 °C. The capillary and cone voltages were optimized to 3.5 kV and 35 V, respectively. Both Q1 and Q3 quadrupoles were set to unit mass resolution. The collision cell pressure was 1.2×10^{-3} mbar. Argon (99.999%) was used as the collision gas for all CID experiments. Collision energies for individual compounds varied from 17 to 23 eV. All mass spectra were recorded in the MCA (multichannel analysis) mode and represent the summation of 10 individual scans. Samples were dissolved in methanol, deionized water or deuterium oxide at a concentration of 50 pmol μl^{-1} and delivered via a syringe infusion pump (Model 200, KD Scientific, Boston, MA, USA) at a flow-rate of 10 $\mu l\ min^{-1}$ into the ion source through a six-port injector (Rheodyne, Cotati, CA, USA) using a 100 μl sample loop.

RESULTS AND DISCUSSION

The general fragmentation pathways for cocaine and its metabolites are shown in Scheme 1. The pyrolysis products AECG and AEME are shown in Scheme 2. Table 1 shows the various R groups represented in Schemes 1 and 2.

CID mass spectrum of protonated cocaine

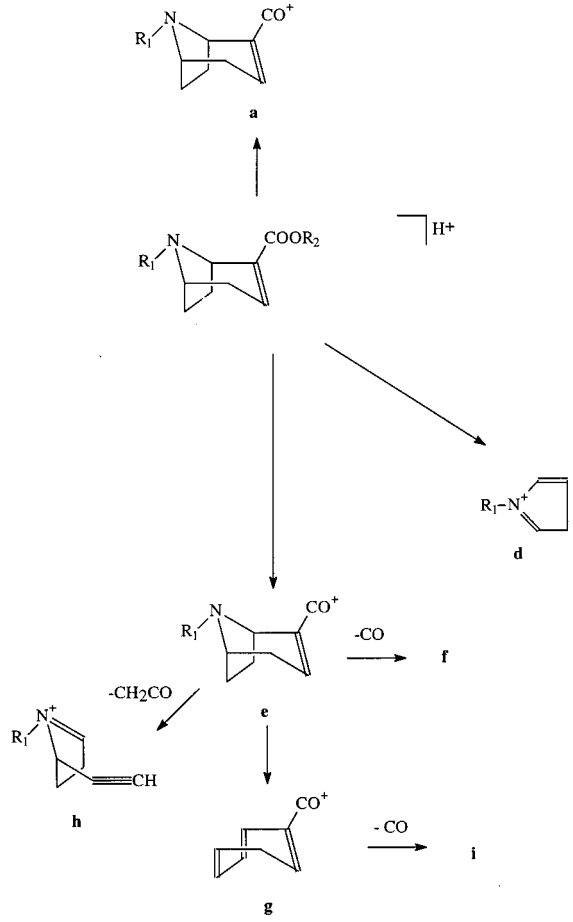
The CID mass spectrum of protonated cocaine is shown in Fig. 1. The assignments of the fragment ions were based on corresponding mass shifts observed for D_3-COC, D_3-BE, D_3-EME and the other metabolites and pyrolysis products. Detailed proposed fragmentation mechanisms for the major fragment ions are shown in Scheme 3. The most abundant fragment ions in all the



Scheme 1. General fragmentation pathway of protonated cocaine and its metabolites.

mass spectra are *m/z* 182 (**c**) which results from the loss of C_6H_5OH and *m/z* 105 (**b**) ($C_6H_5CO^+$).[3] The remaining fragment ions are probably produced by subsequent fragmentation of the ion observed at *m/z* 182, since the fragment ions of *m/z* 82 (**d**), 150 (**e**), 122 (**f**), 119 (**g**), 108 (**h**) and 91 (**i**) were observed in the CID mass spectrum (data not shown) of the ion at *m/z* 182 generated by nozzle–skimmer dissociation. Table 2 lists the *m/z* values and the relative abundances of the major fragment ions from cocaine and related compounds. The formation of fragment ions **d**, **g** and **h** involves the elimination of part of the bicyclo ring system and are thought to involve hydrogen rearrangement reactions.[4,5] Fragment ion **h** is also probably formed through the tautomer of fragment ion **e** and subsequent hydrogen rearrangement.

Ion **d** is a major fragment ion with two possible pathways (Scheme 3(d)) for formation. The first possible mechanism is that fragment ion **d** is formed directly from protonated cocaine through a hydrogen rearrangement (top pathway). Fragment ion **d** could alternatively be derived from fragment ion **c**. To investigate this fragmentation mechanism further, cocaine was dissolved in deuterium oxide (D_2O). Deuterated cocaine (*m/z* 305) was selected to undergo CID experiments. In the first pathway, the resulting fragment ion **d** should have an *m/z* value of 83 instead of 82 in the resulting CID mass spectrum of the $(M + D)^+$ ion. However, in the second pathway, the resulting fragment ion **d** should have the same *m/z* value of 82 because the deuterium



Scheme 2. General fragmentation pathway of protonated cocaine pyrolysis products.

atom is not involved in this mechanism. The result is that fragment ion **d** has the same *m/z* value of 82 in both the protonated and deuterated CID mass spectra ,which indicates that the second fragmentation mechanism is more likely than the first. Fragment ions **g, h** and **i** are minor in the CID mass spectrum of cocaine. Their identities were confirmed by comparing the CID spectra of all the metabolites. Proposed mechanisms for the formation of these ions are shown in Scheme 3(e) and (f). Most likely, hydrogen rearrangements are involved in the formation of these ions.

Discussion of proposed mechanisms

It is important to remember that CID mass spectra result from the dissociation of activated ions, which have more states and structures available to them than do the corresponding unactivated ions. Therefore, proton transfer reactions are possible between the functional groups of the molecules involved in this study (i.e. the four oxygen atoms and the nitrogen atom). Fragment ions **b** and **c** may be generated through similar pathways, either through protonation of the nitrogen or an oxygen atom (Scheme 3(b) and (c)). In the pathway involving protonation of the bridgehead nitrogen, a chair to boat conformation transformation must occur. This conformational change allows for the formation of a transient six-membered transition-state structure before bond breakage. Since the chair to boat conformation transition energy barrier is only 7 kcal mol^{-1} (1 kcal = 4.184 kJ)[6] and is much lower than the collision energy applied (53 kcal mol^{-1} in the center-of-mass frame of reference), this transformation is easily obtained. Fragment ion **a** may also be generated from a selected precursor ion with protonation on either the oxygen of the methyl ester linkage or through protonation of the bridgehead nitrogen with the subsequent formation of a six-membered transition state (Scheme 3(a)).

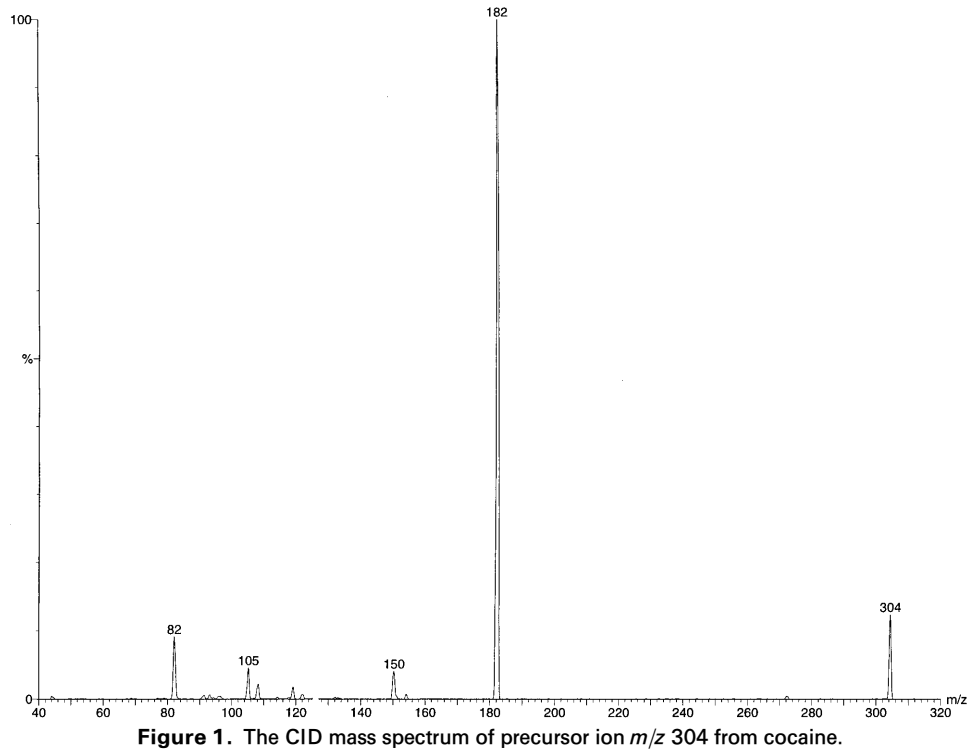
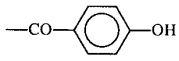
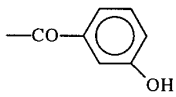
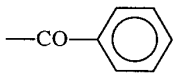
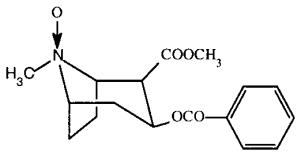
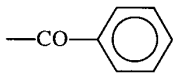
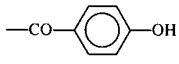
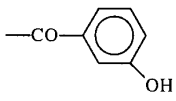
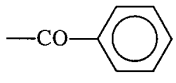
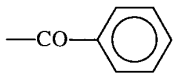
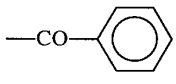
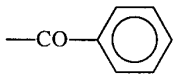
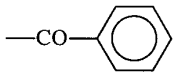
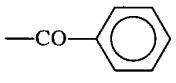
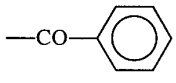
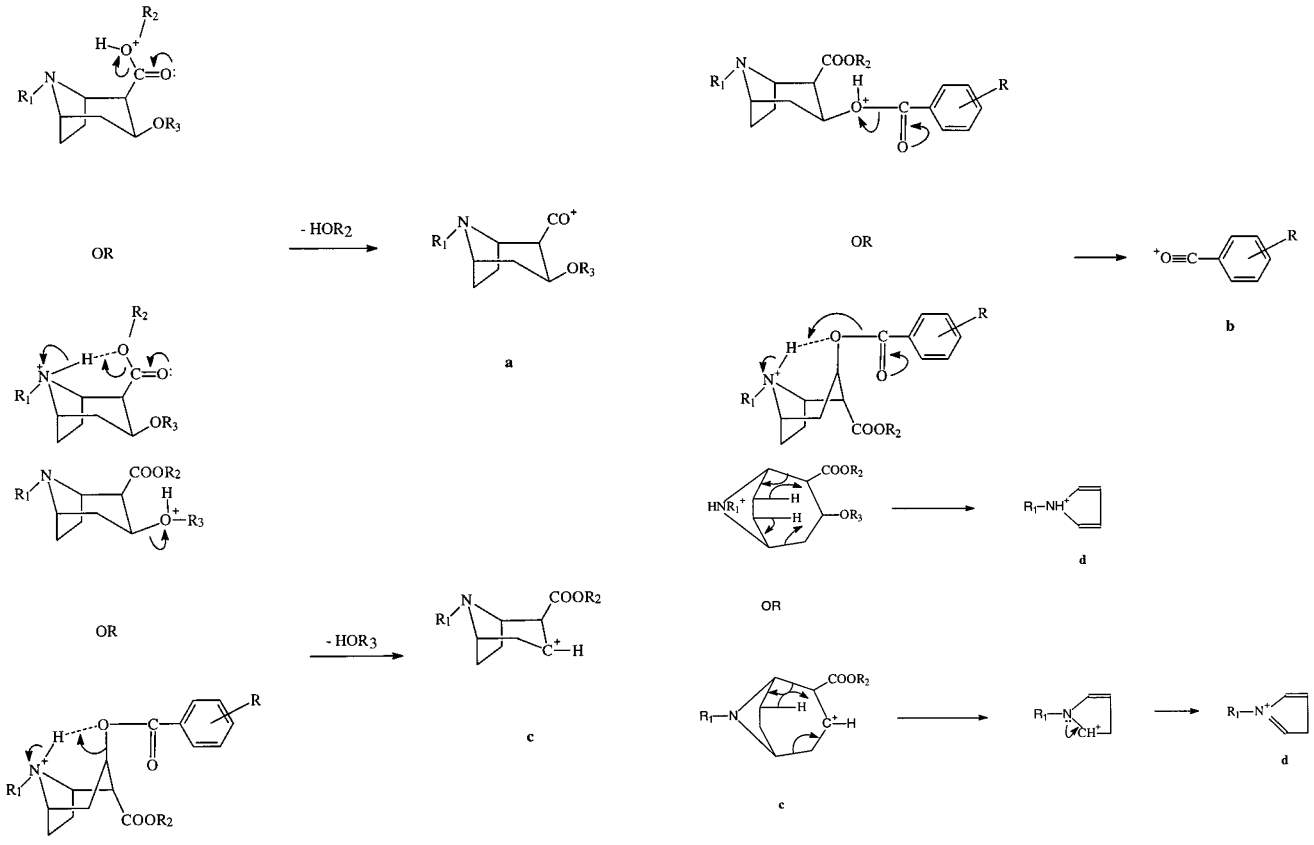


Figure 1. The CID mass spectrum of precursor ion *m/z* 304 from cocaine.

Table 1. The Structure of Cocaine, its Metabolites and Pyrolysis Products

Compounds*	*m/z*, $(M+H)^+$	R_1	R_2	R_3
PHOCOC	320	CH_3	CH_3	—CO—  —OH
MHOCOC	320	CH_3	CH_3	—CO—  OH
CNO**	320	CH_3	CH_3	—CO— 
				
CE	318	CH_3	CH_3CH_2	—CO— 
PHOBE	316	CH_3	H	—CO—  —OH
MHOBE	316	CH_3	H	—CO—  OH
COC	304	CH_3	CH_3	—CO— 
D3COC	307	CD_3	CH_3	—CO— 
NCE	304	H	CH_3CH_2	—CO— 
BE	290	CH_3	H	—CO— 
D3BE	293	CD_3	H	—CO— 
NC	290	H	CH_3	—CO— 
BN	276	H	CH_3	—CO— 
EEE	214	CH_3	CH_3CH_2	H
EME	200	CH_3	CH_3	H
D3EME	203	CD_3	CH_3	H
ECG	186	CH_3	H	H
AEME***	182	CH_3	CH_3	—
AECG***	168	CH_3	H	—

* For abbreviation of the compounds, see experimental section; ** R1, R2 and R3 are same as cocaine except an oxygen atom is attached to the N atom. *** Refer to scheme 2.



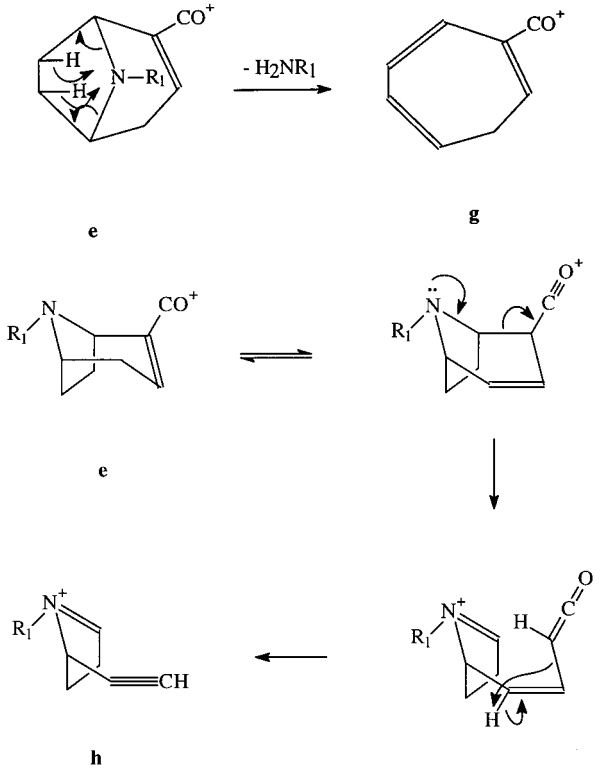
Scheme 3. Proposed fragmentation mechanisms for major fragmentation ions.

The CID mass spectra of the pyrolysis products AECG and AEME still showed the same type of fragment ions as cocaine and its metabolites (except fragment ion **b**). However, the abundance distribution of the fragment ions is different. Minor fragment ions from the CID mass spectra of cocaine and its metabolites, such as **f**, **g** and **i**, are the major ions in the CID mass spectra of pyrolysis products. We hypothesize that the formation of fragment ions **f**, **g** and **i** in the CID mass spectrum of cocaine involved two steps: derived from

Table 2. The mass-to-charge ratios and intensities of fragment ions

	Fragment Ion								
Compound	a	b	c	d	e	f	g	h	i
COC	272(1)	105(5)	182(100)	82(10)	150(6)	122(1.5	119(2)	108(3)	91(0.5)
D3COC	275(1)	105(5)	185(100)	85(10)	153(6)	125(1)	119(0.5)	111(6)	91(0.5)
COC in meconium	272(1.5)	105(4)	182(100)	82(9)	150(6)	122(1.5)	119(3)	108(4.5)	91(2)
PHOCOC	288(0.5)	121(5)	182(100)	82(10)	150(6)	122(1.5)	119(1.5)	108(2.5)	91(0.5)
MHOCOC	288(0.5)	121(6)	182(100)	82(10)	150(7)	122(1.5)	119(1.5)	108(3)	91(1.5)
CNO	288(0.5)	105(15)	198(35), 182(55)*	82(100)*	166(8), 150(2)*	138(6), 122(9)*	119(0.5)	124(0.5), 108(3)*	91(0.5)
CE	272(2)	105(1)	196(100)	82(5)	150(3)	122(0.5)	119(0.5)	108(2)	91(0.5)
PHOBE	288(1)	121(20)	168(100)	82(5)	150(6)	122(0.5)	119(2)	108(0.5)	91(0.5)
MHOBE	288(1)	121(20)	168(100)	82(6)	150(6)	122(1)	119(1.5)	108(0.5)	91(1.0)
NCE	258(0.5)	105(1)	182(100)	68(1)	136(20)	108(1)	119(0.5)	94(0.5)	91(0.5)
BE	272(2)	105(12)	168(100)	82(8)	150(7)	122(1)	119(2)	108(0.5)	91(0.5)
D3BE	275(3)	105(5)	171(100)	85(2)	153(3)	125(0.5)	119(0.5)	111(0.5)	91(0.5)
NC	258(15)	105(42)	168(100)	68(3)	136(25)	108(3)	119(3)	94(3)	91(0.5)
BN	258(0.5)	105(10)	154(100)	68(0.5)	136(45)	108(2)	119(0.5)	94(0.5)	91(0.5)
EEE	168(12)	—	196(100)	82(70)	150(10)	122(3)	119(3)	108(9)	91(0.5)
EME	168(3)	—	182(80)	82(12)	150(3)	122(2)	199(2)	108(2)	91(0.5)
D3EME	171(2)	—	185(72)	85(12)	153(2)	125(1)	119(1)	108(0.5)	91(0.5)
ECG	168(100)	—	168(100)	82(30)	150(5)	122(2)	119(4)	108(2)	91(0.5)
AEME	150(20)	—	182(1)	82(12)	150(20)	122(50)	119(20)	108(0.5)	91(0.5)
AECG	150(10)	—	168(100)	82(10)	150(10)	122(30)	119(20)	108(0.5)	91(0.5)

Note: The values in the bracket represent the relative abundance with base ion intensity 100%.
* Represents the loss of the oxygen atom from this fragment.



Scheme 3 (continued).

fragment ion **c**, then **e**. However, in the CID mass spectra of the pyrolysis products, only one step is involved directly from fragment ion **e**. The shorter pathway facilitates the formation of these fragment ions.

CID mass spectrum of cocaine *N*-oxide

Misra *et al.*[7] first reported the presence of cocaine *N*-oxide (CNO) in 1979. Although it showed high liver toxicity, there is no report of its direct measurement in either humans or animals following administration of cocaine. The reason is that most determinations of cocaine and its metabolites are performed using GC/MS and this metabolite is thermally labile. We recently reported the observation and quantitation of CNO in the rat following i.v. administration of cocaine and in human meconium samples from cocaine-exposed neonates using an LC/MS method.[2]

The CID mass spectrum of protonated cocaine *N*-oxide (CNO) is worthy of discussion. CNO has lower stability upon collisional activation with neutral argon gas and the oxygen atom attached to the bridgehead nitrogen can be easily stripped away. This instability leaves the CID mass spectrum of protonated CNO looking similar to that of cocaine (see Table 2, CID mass spectrum of CNO not shown). Almost all major *N*-containing fragment ions (*m/z* 182 (**c**), 82 (**d**), 150 (**e**), 122 (**f**) and 108 (**h**), except 272 (**a**) which is a very minor fragment ion in this system) observed in the CID mass spectrum of cocaine were observed in the CID mass spectrum of CNO. It is worth noting that in the CID mass spectrum of CNO the ion at *m/z* 82 is formed exclusively and its oxygen attached counterpart, at *m/z* 98, was not observed. Further CID studies of CNO applying higher collision energies showed that the oxygen atom attached to the bridgehead nitrogen was removed more completely, resulting in an increase in the abundances of the fragment ions identical with those observed for COC.

CONCLUSION

A detailed study of the fragmentation pathways for cocaine and its metabolites and related pyrolysis products has been presented. The assignments of the fragment ions were supported by mass shifts from deuterium-labeled cocaine, benzoylecgonine and ecgonine methyl ester. Additional support was gained for several mechanisms by comparing the CID mass spectra of protonated cocaine (*m/z* 304) and deuterium-exchanged cocaine (*m/z* 305).

An understanding of the fragmentation of cocaine and its related compounds is essential to any future attempts to quantitate these compounds in biological matrices. This study showed that the transition to be monitored for an LC/MS/MS assay of a majority of the compounds studied would be from the protonated molecular ion to fragment ion **c**. In the case of CNO the fragment ion that should be monitored was **d** at *m/z* 82 and for the pyrolysis product AEME fragment ion **f** at *m/z* 122 was observed to be the most abundant at the selected collision energy.

A final question to be addressed is whether it will be necessary to use LC/MS/MS, as opposed to LC/MS, for the quantitation of these compounds in biological matrices. The choice of LC/MS is attractive owing to its greater simplicity (fewer optimizable parameters) and the need for only a single mass analyzer, therefore making it a more widely applicable assay. For assays employing electrospray ionization, generally only the intact protonated molecular species is observed. Since there is little fragmentation, the sensitivity of this method can be good because the ions representing the analyte are concentrated in a single peak. If additional fragment peaks are desired for further analyte verification, these may be obtained through in-source CID (nozzle–skimmer dissociation). The use of in-source CID will decrease the overall sensitivity of the assay, since the ions that were concentrated in a single peak are now spread over a number of peaks. LC/MS/MS provides greater specificity than LC/MS since the mass-selected precursor and product ions must both be present for detection to occur. In addition, LC/MS/MS may provide greater sensitivity (in terms of signal-to-noise ratio) if there is a high level of chemical noise in the primary mass spectrum. In this case MS/MS acts as a filter removing interfering chemical noise. It is this ability to remove interfering background ions that provides LC/MS/MS with such an advantage over LC/MS. The reduction in chemical noise using LC/MS/MS has led to the development of fast chromatography, which can be used to improve the sample throughput dramatically.

In the case of cocaine and its metabolites and pyrolysis products, the development of an all-encompassing LC/MS(/MS) assay will be challenging. The presence of the *meta*-and *para*-hydroxylated isomers makes their

chromatographic separation a necessity, as they cannot be distinguished based on their tandem mass spectra. However, the major hurdle to be overcome in the development of a quantitative assay for these compounds will not be the mass spectrometry or the chromatography but the extraction procedure. It will be difficult to find a method able to achieve high recoveries for compounds having such a wide range of polarities.

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