

The Mass Spectrum of Cocaine: Deuterium Labeling and MS/MS Studies

R. Martin Smith*

rMS Workshops, LLC
6442 Dylun Drive
Madison, WI 53719

[email: rmsworkshopsllc -at- msn.com]

John F. Casale

U.S. Department of Justice
Drug Enforcement Administration
Special Testing and Research Laboratory
22624 Dulles Summit Court
Dulles, VA 20166

[email address withheld at author's request]

ABSTRACT: Seven derivatives of cocaine (*l*-2-*exo*-carbomethoxy-3-*exo*-benzoyloxytropane; **1a**) were synthesized in which the hydrogen (H) atoms at various positions were replaced by deuterium ($^2\text{H} = \text{D}$) – specifically, at the N-methyl, O-methyl, phenyl, 2-, 3-, and 4- positions, as well as at the combined 1-, 5-, 6-, and 7- positions (**1b** - **1h**, respectively). The mass spectra of these compounds were recorded. Elemental compositions for selected ions in the spectrum of **1a** were determined using high-resolution mass spectrometry, and precursor and product ion spectra for many of these ions were studied using MS/MS. Mechanisms for many previously proposed fragmentation pathways were either confirmed or clarified, and new insights were gained into the fragmentation of **1a**. Reasons for variations in relative intensities of the m/z 94 and 152 peaks between the spectra of the cocaine diastereomers are proposed.

KEYWORDS: forensic science, cocaine, mass spectrometry, fragmentation mechanisms, deuterium-labeled derivatives, high-resolution MS, MS/MS, product and precursor ions, pseudococaine.

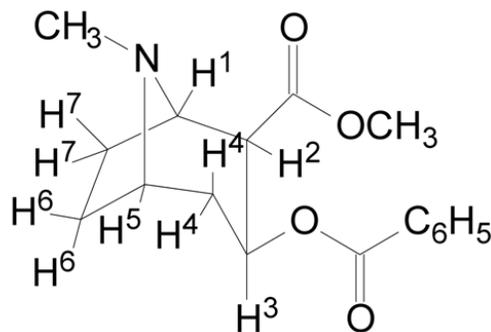
The electron ionization (EI) mass spectra of tropane derivatives were first studied by Budzikiewicz, Djerassi, and coworkers, using the spectra of deuterated derivatives and the examination of metastable ion spectra [1,2]. Cocaine (*l*-2-*exo*-carbomethoxy-3-*exo*-benzoyloxytropane; **1a**), a molecule of considerable forensic interest, was not included in the original studies. Subsequent workers elucidated similar basic fragmentation pathways for **1a** (Figures 1-3) [3,4,5]. Many questions remain, however, regarding the nature of some fragmentations and the formation of less abundant ions. In particular, previous proposals concerning the relative intensities of the m/z 152 peak and the m/z 94/96 pair of peaks in the spectra of the cocaine diastereomers seem unsatisfactory. The present work attempts to expand upon this knowledge.

Seven labeled derivatives of **1a** were prepared in which the hydrogen (H) atoms at specific positions were replaced by deuterium ($^2\text{H} = \text{D}$; structures **1b**-**1h**). Mass spectra for these compounds were recorded and examined to determine the presence or absence of the labels for many of the ions represented in the cocaine spectrum. In addition, MS/MS data were collected in both the precursor (“parent”) and product (“daughter”) ion modes in order to ascertain relationships between the various ions.

Experimental Procedures

Solvents, Chemicals, and Materials

All solvents were distilled-in-glass products of Burdick and Jackson Laboratories (Muskegon, MI). N-Methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) was obtained from Pierce Chemical (Rockford, IL). All other chemicals were



Cocaine (**1a**: All H = H)

O-CD₃ (**1b**: O-CH₃ = OCD₃; all other H = H)

NCD₃ (**1c**: N-CH₃ = N-CD₃; all other H = H)

Phenyl-*d*₅ (**1d**: C₆H₅ = C₆D₅; all other H = H)

2-*d*₁ (**1e**: H² = D; all other H = H)

3-*d*₁ (**1f**: H³ = D; all other H = H)

4,4-*d*₂ (**1g**: H⁴ = D; all other H = H)

1,5,6,6,7,7-*d*₆ (**1h**: H¹ = H⁵ = H⁶ = H⁷ = D; all other H = H)

reagent grade quality products of Sigma-Aldrich Chemical (Milwaukee, WI). Alumina (basic) was deactivated slightly by adjusting the water content to 4% (w/w). Cocaine (**1a**), pseudococaine, ecgonine methyl ester, N-trideuteriomethylcocaine (**1c**), and α -cocaine were from the authentic reference collection of the DEA Special Testing and Research Laboratory.

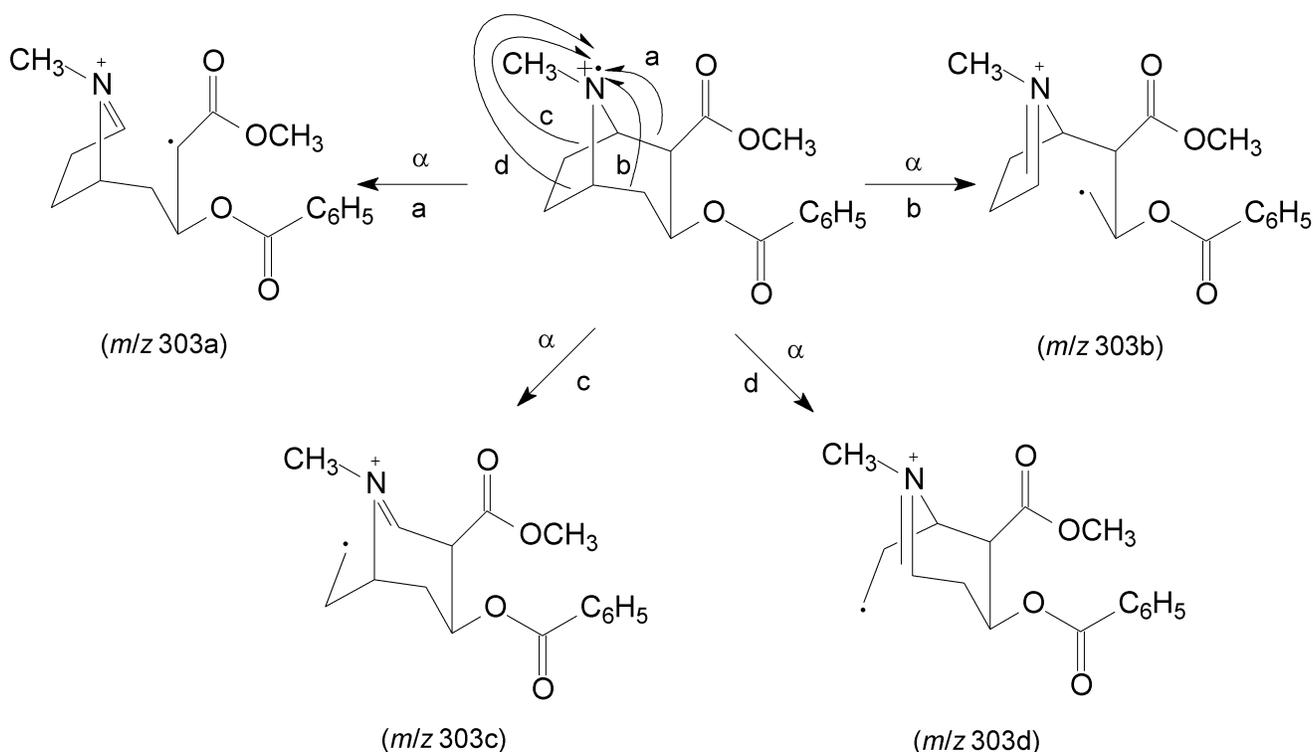


Figure 1 - α -Cleavage fragmentations of the cocaine molecular ion after initial ionization at N.

Synthesis

All syntheses were performed in flame-dried glassware and protected from moisture. **Ie**, **If**, **Ig**, **Ih**, and 2- d_1 -, 3- d_1 -, 4,4- d_2 -, 1,5,6,6,7,7- d_6 -pseudococaine were synthesized as previously described [6]. Unlabelled 2-carbomethoxy-3-tropinone was also synthesized as previously described [7]. Yields for the following syntheses were not optimized.

Cocaine (OCD₃) (Ib): Anhydrous benzoylecgonine (110 mg, 0.380 mmol) was combined with CH₂Cl₂ (4 mL) and 1',1'-carbonyldiimidazole (63.5 mg, 0.391 mmol) in a 15 mL centrifuge tube. The reaction was allowed to stand overnight. Trideuteriomethanol (CD₃OH; 100 μ L) was added and the mixture allowed to stand for one day. The reaction mixture was evaporated to dryness under a stream of nitrogen at 75°C and treated with 7 mL of hot hexane. The hexane was decanted to a new tube and allowed to cool, precipitating the imidazole by-product. The hexane was filtered, evaporated *in vacuo*, recrystallized from hexane, and dried to provide a white powder (42 mg, 36% yield).

Cocaine (phenyl- d_5) (Id): Ecgonine methyl ester hydrochloride (500 mg, 2.12 mmol) was suspended in 7 mL of dry pyridine in a 100 mL round bottom flask, to which pentadeuteriobenzoyl chloride (437 mg, 3.00 mmol) was added. After stirring for 5 days, the reaction was diluted with 50 mL of dry acetone to precipitate the product. The crude product was captured by suction filtration, dissolved into water (4 mL), rendered alkaline (pH = 9) with aqueous Na₂CO₃, extracted with CHCl₃ (1 x 8 mL), dried over anhydrous Na₂SO₄, filtered, and evaporated *in vacuo* to a crystalline mass. The product was recrystallized from hexane to provide a white powder (114 mg, 17% yield).

Anhydroecgonine methyl ester: Cocaine base (7.1 g, 23.4 mmol) was refluxed in 6N HCl overnight, and then evaporated to a residue on a steam bath. The residue was transferred to a

flask containing MeOH (50 mL) and boron trifluoride diethyl etherate (1.0 g, 7.05 mmol) and refluxed for 2 hours. The reaction was evaporated *in vacuo*, quenched with water (40 mL), adjusted to pH 9 with Na₂CO₃, extracted with CHCl₃ (2 x 100 mL), dried over anhydrous Na₂SO₄, filtered, and evaporated *in vacuo* to a semi-crystalline mass. Approximately 1.5 g of the crude product was dissolved in a minimal amount of Et₂O and loaded onto a glass chromatographic column (1 x 22 cm) containing 15 g of basic alumina (150 mesh). The column was eluted sequentially with 20 mL each of the following solvents: 1) Et₂O, 2) Et₂O/CHCl₃ (1:1), and 3) CHCl₃. Ten mL fractions were collected and examined by GC/MS, both underivatized and following derivatization with MSTFA. The first three fractions were combined and evaporated to dryness to give a clear oil (655 mg, 15% yield).

2,3-Dehydrococaine: 2-Carbomethoxy-3-tropinone (153 mg, 0.776 mmol) was combined with dry pyridine (2 mL) and benzoyl chloride (300 mg, 2.13 mmol) in a 15 mL centrifuge tube and left to stand for 1 hour. Et₂O (13 mL) was added to precipitate the crude product, which was washed with additional Et₂O (10 mL). The semi-crystalline material was dissolved in 0.36N H₂SO₄ (1 mL), washed with Et₂O (2 x 14 mL), adjusted to pH 9 with Na₂CO₃, extracted with CHCl₃ (1 x 10 mL), dried over anhydrous Na₂SO₄, filtered, and evaporated *in vacuo* to give an off-white powder (118 mg, 50% yield).

Gas Chromatography/Low Resolution-Mass Spectrometry (GC/LR-MS)

GC/LR-MS analyses were performed using an Agilent (Palo Alto, CA) Model 5973 quadrupole mass-selective detector (MSD) interfaced with an Agilent Model 6890 gas chromatograph. The GC system was fitted with a 30 m x 0.25 mm ID fused-silica capillary column coated with DB-1 (0.25 μ m) (J & W Scientific, Rancho Cordova, CA). The oven

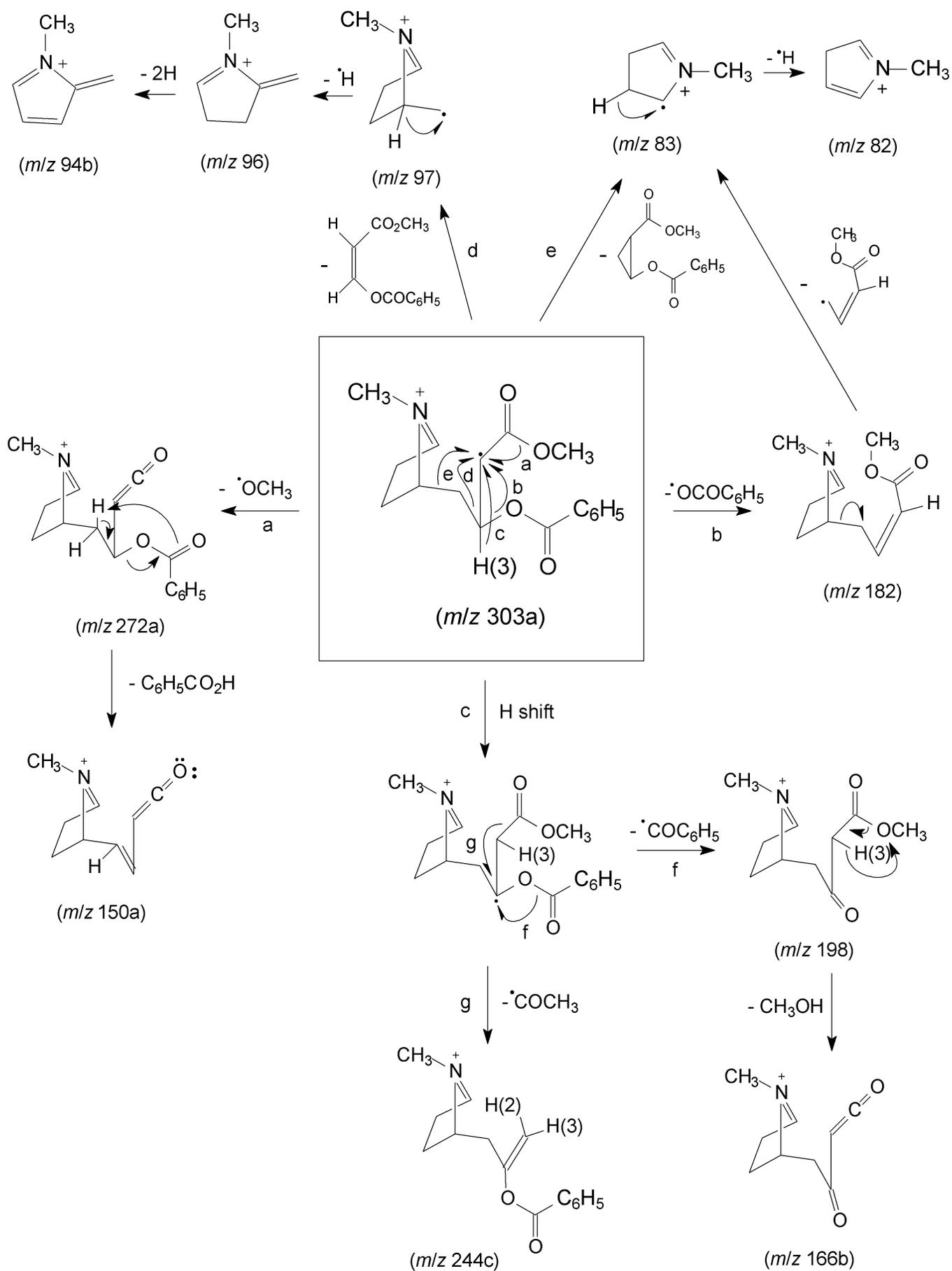


Figure 2 - Previously proposed fragmentations of **1a** following initial *alpha*-cleavage next to the N atom.

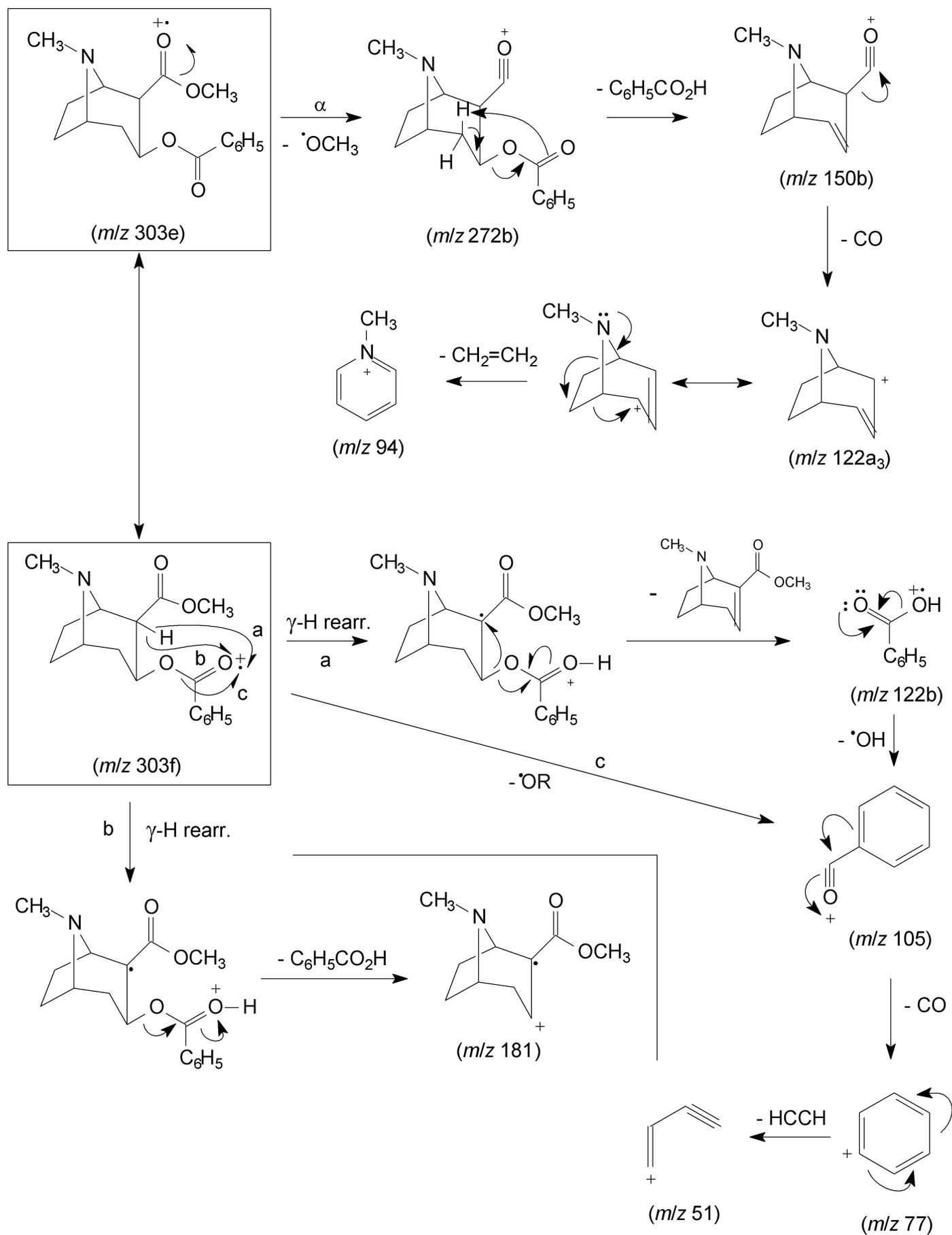


Figure 3 - Previously proposed fragmentations of **1a** following initial ionization at the ester O atoms.

temperature was programmed as follows: Initial temperature, 100°C; initial hold, 0.0 min; program rate, 6°C/min; final temperature, 300°C; final hold, 5.67 min. The injector was operated in the split mode (21.5:1) at a temperature of 280°C. The MSD was operated in the electron ionization (EI) mode with an ionization energy (I.E.) of 70 eV and a scan range of 34-700 *m/z* units at 1.34 scans/s. The auxiliary transfer line to the MSD and the source were maintained at 280°C and 230°C, respectively.

High Resolution-Mass Spectrometry (HR-MS)

HR-MS data were obtained on two instruments. The first was a Finnigan MAT Model 8230 system (Bremen, Germany) operating at an I.E. of 70 eV. The source temperature was 175°C. The second was an AEI Scientific Apparatus Model MS-902 system (Manchester, UK) operating at an I.E. of 70 eV. The source temperature was maintained at 120-150°C. Sample introduction was accomplished with a solids probe on both systems, and data was acquired at a resolution of *ca.* 10,000 (5% valley).

Precursor/Product Ion Mass Spectrometry (MS/MS)

Precursor and product ion data were obtained on a Thermo Scientific TSQ-7000 triple quadrupole MS system (Bremen, Germany). Ionization energies were varied from 5-70 eV. Sample introduction was accomplished with solids probe. Unfortunately, these data were obtained several years ago without details of the conditions used being recorded. These instruments are no longer in the authors' laboratories.

Results and Discussion

Electron ionization mass spectra of **Ia** and of the seven D-labeled derivatives (**Ib-Ih**) are shown in Figures 4-6. Careful examination and comparison of these spectra reveal intensity patterns that are reasonably consistent from one spectrum to the next. The positions of individual peaks within the spectra, however, vary depending upon the presence or absence of the D label(s) in the ion(s) represented. When some or all the D label is retained in a particular ion, the position of the peak corresponding to this ion moves to a higher *m/z* value in the spectrum of the D-labeled compound (the "shift technique") [8]. The number of D atoms retained is determined by the difference in *m/z* value between the peak in the cocaine spectrum and that in the spectrum of the D-labeled derivative.

A compilation of *m/z* values for selected peaks in the spectrum of **Ia** and for the corresponding peaks in the spectrum of each of the derivatives is shown in Table I. In many cases, each peak in the spectrum of **Ia** can be correlated to a single peak in the spectrum of each derivative. When this occurs, a single fragmentation mechanism may account for formation of that ion.

In some cases, especially for lower *m/z* peaks, a single peak in the spectrum of **Ia** is represented by two or more peaks in a derivative spectrum. This indicates that two or more mechanisms may be operative in forming that ion, or that a single peak in the low-resolution spectrum may represent two or more ions. Given the multiplicity of potential precursors for many low *m/z* ions, this is not surprising. In a few instances, the peak correspondences between spectra are either too complex to be determined with confidence or suffer from interferences from

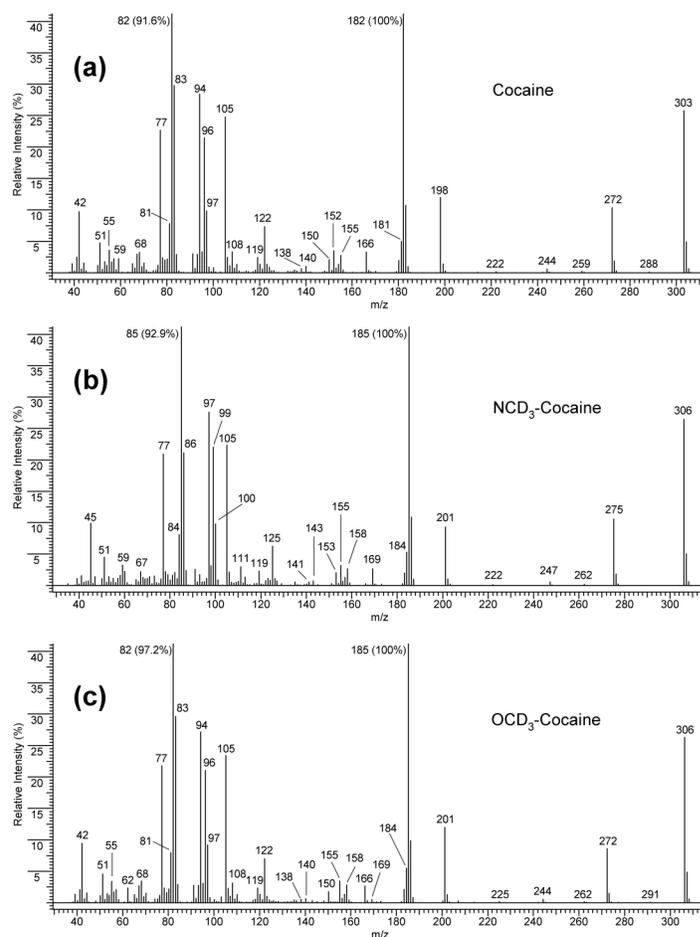


Figure 4 - Electron ionization mass spectra of a) cocaine (**Ia**); b) N-CD₃-norcocaine (**Ic**); and c) benzoylecgonine trideuteriomethylester (**Ib**).

nearby peaks. These instances are noted in Table I.

Table II summarizes the elemental compositions of ions formed during the fragmentation of **Ia** as determined by HR-MS. The elemental compositions confirm those determined by Shapiro, *et al.* [9], although the list presented here is more extensive. Table II also lists precursor and product ions for these ions as identified by MS/MS. These data represent a substantial condensation of the raw data. In order to best identify precursor-product ion relationships, emphasis was placed on scans at the lowest collision energies (5-10 eV) because product ions formed under these conditions are the least likely to undergo additional fragmentation. A schematic representation of these relationships is shown in Figure 7. Although it is tempting to use the information in Figure 7 to define EIMS fragmentation pathways for **Ia**, it must be remembered that both precursor and product ions formed by collisional activation in the MS/MS experiments may have different structures than those formed during EIMS [10].

The molecular ion (M^+) of **Ia** ($C_{17}H_{21}NO_4^+$, *m/z* 303) fragments at collision energies of 5-20 eV to produce at least nine stable product ions that apparently form without the detectable intermediacy of other ions. These include the listed ions having *m/z* 272, 222, 198, 182, 181, 122, 97, 83, and 82 (Table III). Although product ion scans for *m/z* 303 did not produce peaks at *m/z* 288, 275, 274, 259, 244, and 155 under these conditions, other data indicate that in the EI spectrum these ions may also

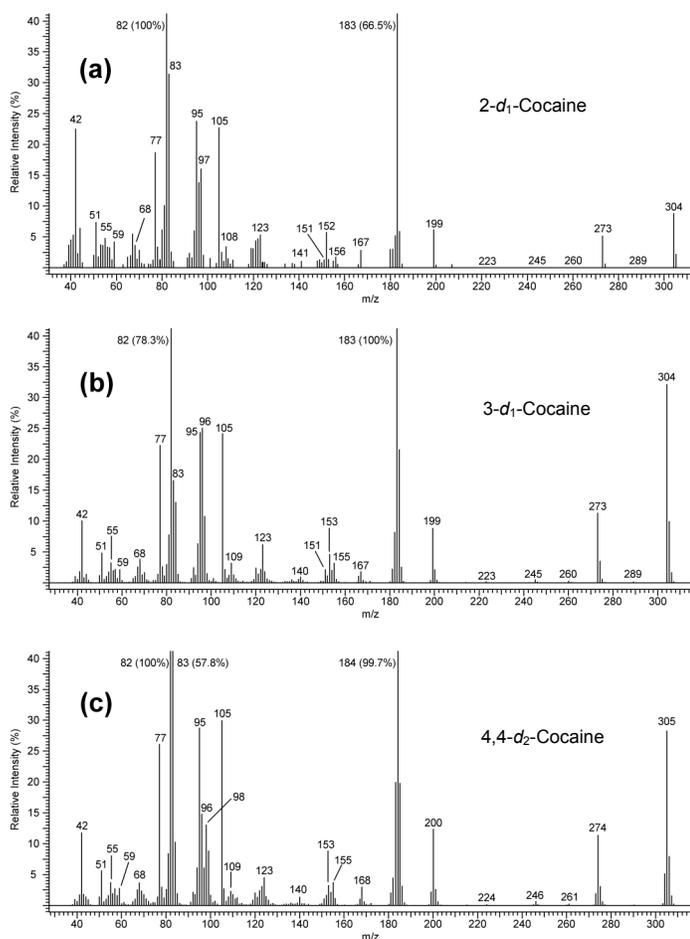


Figure 5 - Electron ionization mass spectra of a) 2-deuteriococaine (**Ie**); b) 3-deuteriococaine (**If**); and 4,4-dideuteriococaine (**Ig**).

form directly from m/z 303 (see below).

The most abundant ions formed from the M^+ at 5-10 eV in the MS/MS experiments are those with m/z 198, 181, 83, and 82. At the collision energies listed in Table III, the m/z 182 ion accounts for only a peak of 2-4% relative intensity, which seems at odds with the fact that the m/z 182 peak is usually one of the two most intense peaks in the 70 eV EI spectrum. However, it must be remembered that peaks observed in EIMS result from the interplay of a number of factors – most importantly, the percentage of precursor ion(s) that produce the represented ion, the internal energies of both precursor and product ions, and the stability of the product ion toward further fragmentation [11]. Thus, even though relatively few m/z 303 ions appear to form m/z 182 ions directly at low collision energies, this ion is listed as a precursor ion for only one other relatively abundant ion represented in the EI spectrum (m/z 82). The m/z 182 ions formed even under the more energetic EIMS conditions seem relatively resistant to additional fragmentation, leading to a larger percentage of m/z 182 ions surviving the journey from the ion source through the analyzer to the detector.

The combined labeling and HR-MS data allow postulation of meaningful pathways for the fragmentations of cocaine ions. In making these proposals, a few guidelines were followed: a) formation of the M^+ is assumed to take place by removing a non-bonding electron from the nitrogen (N) or oxygen (O)

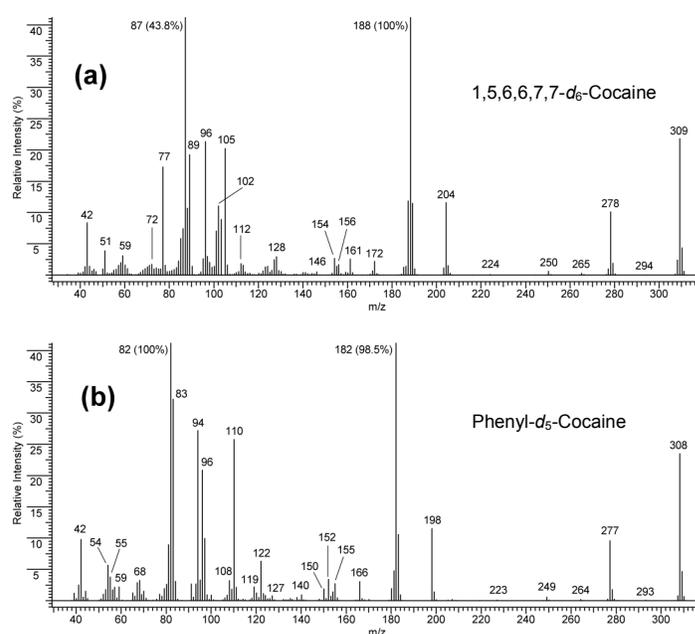


Figure 6 - Electron ionization mass spectra of a) 1,5,6,6,7,7-hexadeuteriococaine (**Ih**); and b) pentadeuteriobenzoylmethylcogonine (**Id**).

atoms, rather than those within the carbon framework (see Figures 1-3, for example); b) structures containing highly strained bonds were deemed less probable as structures for ions if lower-energy alternative structures could be proposed; and c) ions known to have low ionization energies (I.E.) of formation, such as carbonyl and iminium ions [12], were preferred whenever possible. Because of the low I.E. for the n -electrons on the N atom and the stability of the resulting ions, the most significant fragmentations following initial ionization are the α -cleavages shown in Figure 1. Of these, m/z 303a should be the most stable because the radical site is stabilized by conjugation with the double bond in the carbonyl group; whereas, no conjugation is possible in the other structures. Even though their lifetimes are undoubtedly very short, the ions formed in these first fragmentations serve as precursors to many of the product ions discussed below.

m/z 288. The weak m/z 288 peak reflects a loss of 15 u - a methyl radical - from the M^+ . The only D label lost in this fragmentation is that on the N-CH₃ group (Table I). The mechanism proposed in Figure 8 for its formation generates conjugated double bonds and places the final charge on the N atom. At low collision energies, this ion is one of several precursors to the m/z 166 ion (Table II). The additional loss of the phenyl ring and a H atom at C4 (shown in Figure 8) is consistent with the D labeling pattern for one of the m/z 166 ions (see below).

m/z 275. No fragment ion peak is usually observed in the EI spectrum at m/z 275. However, an ion having this m/z value is implicated as a potential intermediate during the formation of the m/z 154 ion (see below). Its intermediacy in the formation of some m/z 274 ions cannot be ruled out (see subsequent discussion).

m/z 274. It is easy to assume that the small peak at m/z 274 is

Table I - Peak Correspondence Table.

Cocaine peak <i>m/z</i>	O-CD ₃ <i>m/z</i>	N-CD ₃ <i>m/z</i>	Phenyl- <i>d</i> ₅ <i>m/z</i>	2- <i>d</i> ₁ <i>m/z</i>	3- <i>d</i> ₁ <i>m/z</i>	4,4- <i>d</i> ₂ <i>m/z</i>	1,5,6,6,7,7- <i>d</i> ₆ <i>m/z</i>
303	306	306	308	304	304	305	309
288	291	288	293	289	289	290	294
274	277	277	279	275	275	275,276	276
272	272	275	277	273	273	274	278
259	262	262	264	260	260	261	265
244	244	247	249	245	245	246	250
222	225	222	227	223	223	224	224
198	201	201	198	199	199	200	204
182	185	185	182	183	183	184	188
181	184	184	181	181,182	182	182,183	187
166	166,169	(166),169	166	(166),167	166,167	(167),168	(171),172
155	158	158	155	156	155	155,156	161
154	157	157	154	155	154?	154+	155?, 156?
152	155	155	152	152	153	153	154
150	150	153	150	151?	151	151,152	155,156
140	140	143	140	141	140	140	144,145,146
138	138	141	138	139?	139	139,140	140,141?
122a	122	125	122	123	123	(123),124	127,128
122b	122	122	127	122	122	122	122
108	108	111	108	108,109	109	109,110	112,113
105	105	105	110	105	105	105	105
100	103	100?	100	101	101	101,102?	?
97	97	100	97	97?	97	98,99?	102,103?
96	96	99	96	96(-97?)	96	96,98?	96?,101?
94	94	97	94	95	95	95	96?
83	83	86	83	83	83,84?	83,84	88,89
82	82	85	82	82	82	82,83?	87,88?
77	77	77	82	77	77	77	77
68	68	? + 71	68	68	68	68	69-73?
67	67	67 + ?	67	67	67	67 + ?	68-73?
59	62	59	59	59	59	59	59
55	55	58?	55	55	55	55	57-61?
51	51	51	54	51	51	51	51
42	42	45	42	42	42	42	43

Key: -**Boldface** indicates partial or total loss of deuterium label at that position.
 -Numbers in parentheses indicate a peak of much smaller intensity.
 -Question mark (?) indicates that data cannot be interpreted with confidence.

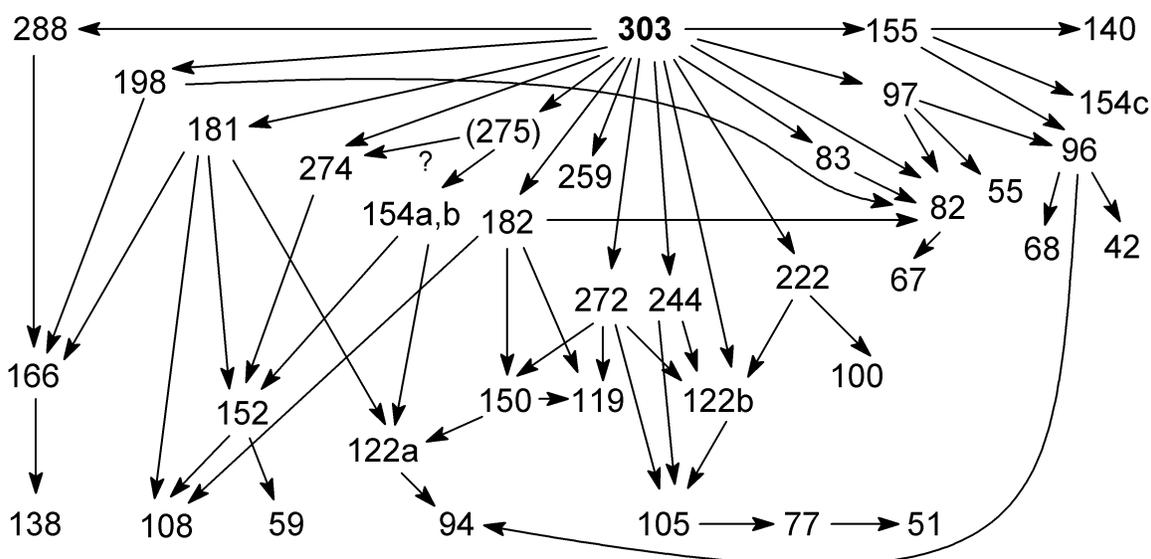
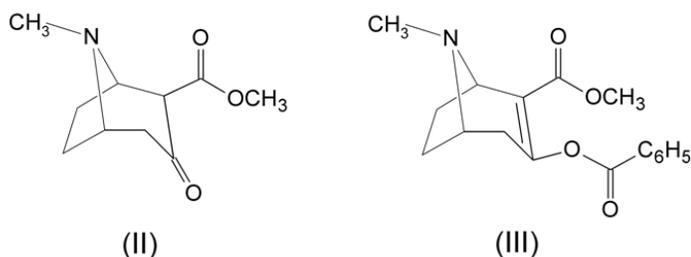


Figure 7 - Relationships between various cocaine fragment ions as determined by MS/MS.

an isotope peak in the m/z 272 ion cluster. Alerted by the MS/MS data that an ion having m/z 274 is a precursor to the m/z 152 ion (Table II), careful analysis reveals that this peak is consistently about 0.3-0.6% too large to be due to isotope contributions alone. The spectra of the D-labeled derivatives shows partial loss of one D at C4 for this peak, plus the loss of four D atoms from positions 1, 5, 6, and 7. Loss of the D label at C2 could not be determined with certainty. Because an ion having m/z 274 reflects the loss of 29 u from the M^+ , and five of these mass units are due to H atoms, the remainder must consist of two C atoms. This loss is most easily envisioned as a H atom from either C2 or C4 plus C6 and C7 with their four attached H atoms (Figures 9 and 10). This may occur via H rearrangement followed by ethyl radical loss [1,13], or by loss of ethylene to produce m/z 275 and subsequent H radical loss from either C2 or C4. In 2-carbomethoxy-3-tropanone (II), 2,3-anhydrococaine (III), and other tropanes having some unsaturation at C2 and/or C3, peaks are observed at both M-28 and M-



29, indicating that, at least in those compounds, both pathways occur [1, 2, 14]. In those cases, loss of 29 u leads to an ion having aromatic character (Figure 11). That is not the case here.

Loss of benzoic acid from m/z 274 produces the m/z 152 ion with additional loss of the D labels on the phenyl ring and either C2 or C4 (see below).

m/z 272. The m/z 272 ion is formed by loss of a methoxy radical from the methyl ester group in the M^+ . This is supported by the elemental composition and labeling data in Tables I and II. As expected, the only D label lost is the one on the OCH_3

group. Two mechanisms for formation are possible that cannot be distinguished using available data (Figures 2 and 3). Both have been proposed previously [3,4,15].

m/z 259. No HR-MS or MS/MS data were recorded for this ion during this study. The loss from the M^+ is 44 u, the mass of CO_2 . All D labels are retained, indicating that no H atoms are lost during this fragmentation. It is impossible to tell from the available data which of the two carboxyl groups is lost. The mechanism in Figure 12 shows loss of the alkyl carboxyl group. Although a stepwise mechanism is shown, a nearly concerted elimination of CO_2 with methyl group migration is possible.

m/z 244. The elemental composition determined for this ion by HR-MS shows loss of $C_2H_3O_2$ from the M^+ . The only D label lost is that on the OCH_3 group, consistent with loss of a carbomethoxy radical.

Formation of m/z 244 is most easily depicted as resulting from m/z 303c or 303d (Figures 9 and 10). Its formation from m/z 303a cannot occur without H rearrangement, and formation from m/z 303b would likely result in either a cyclopropane ring or a diradical. "Backside" attack at C2 by the radical site at C7 in m/z 303c causes elimination of a carbomethoxy radical and generates a bicyclo[2.2.2] ion (Figure 9) [5]. Alternatively, a similar attack by a radical site at C6 produces a bicyclo[4.2.0] ion (Figure 10).

Both proposed m/z 244 ions have fragmentation options that can lead to product ions listed in Table II. Loss of ethylene by a cycloelimination reaction forms m/z 216 (identified as a product ion in the MS/MS data, even though the corresponding peak is not observed in the spectrum). The m/z 244 ion is a possible precursor to the m/z 105 ion, as well as to one or more of the m/z 122 ions [5]. Cyclic loss of benzoic acid or its corresponding radical ion produces m/z 122a₁ and 122a₂ or 122b, respectively; and migration of the charge to the benzoyl O atom generates the benzoyl ion (m/z 105).

m/z 222. This ion, which is usually represented by a peak of very low intensity in the EI spectrum, is formed directly from

Table II - Elemental Composition and MS/MS Table.

Cocaine Ion <i>m/z</i>	Elemental Composition	Fragment Lost	Precursor Ion(s) At 5-15 eV (<i>m/z</i>) ^{a,b}	Important Product Ion(s) At 5-15 eV (<i>m/z</i>) ^{a,c}
303	C ₁₇ H ₂₁ NO ₄	None	None	272,222, 198 ,182, 181 , 83 , 82
288	Not Determined	(CH ₃)	(303)	(166)
274	Not Determined	(C ₂ H ₅)	(303)	(152)
272	C ₁₆ H ₁₈ NO ₃	OCH ₃	(303)	150 , 122, 105, 82
244	C ₁₅ H ₁₈ NO ₂	CH ₃ CO ₂	(303)	216, 122, 105
222	Not Determined	(C ₅ H ₇ N)	(303)	(100)
198	C ₁₀ H ₁₆ NO ₃	C ₆ H ₅ CO	303	166, 82
182	C ₁₀ H ₁₆ NO ₂	C ₆ H ₅ CO ₂	303	150 , 108, 82
181	C ₁₀ H ₁₅ NO ₂	C ₆ H ₅ CO ₂ H	(303)	180,166,152, 122 ,108,94
166	C ₉ H ₁₂ NO ₂	C ₈ H ₉ O ₂	303,288, 198 ,181	138, 134, 110, 82
155	C ₈ H ₁₃ NO ₂	C ₉ H ₈ O ₂	(303)	140, 96 , 82
154	C ₈ H ₁₂ NO ₂	C ₉ H ₉ O ₂	(275), (155)	(152), 122 , 94
152	C ₈ H ₁₀ NO ₂	C ₉ H ₁₁ O ₂	274, 181 ,154	122, 108 , 93, 92 , 59
150	C ₉ H ₁₂ NO	C ₈ H ₉ O ₃	(272), (182)	122 , 119, 93, 91, 82
140	C ₇ H ₁₀ NO ₂	C ₁₀ H ₁₁ O ₂	155	Not Determined
138	C ₇ H ₈ NO ₂	C ₁₀ H ₁₃ O ₂	(166)	Not Determined
122a	C ₈ H ₁₂ N	C ₉ H ₉ O ₄	181 , 150	107, 94 , 91, 81
122b	C ₇ H ₆ O ₂	C ₁₀ H ₁₅ NO ₂	303 ,272,244,222	105 , 77
108	C ₇ H ₁₀ N	C ₁₀ H ₁₁ O ₄	(182),(181),(152)	93 , 42
105	C ₇ H ₅ O	C ₁₀ H ₁₆ NO ₃	272 , 244 ,122	77, 51
100	C ₅ H ₈ O	C ₁₂ H ₁₃ NO ₃	303 , 222	Not Determined
97	C ₆ H ₁₁ N	C ₁₁ H ₁₀ O ₄	303 ,156	96, 82 , 55
96	C ₆ H ₁₀ N	C ₁₁ H ₁₁ O ₄	303, 155 ,97	94 , 81, 79, 68, 42
94	C ₆ H ₈ N	C ₁₁ H ₁₃ O ₄	303,181,154, 122 ,96	93, 78
83	C ₅ H ₉ N	C ₁₂ H ₁₂ O ₄	(303)	82 , 42
82	C ₅ H ₈ N	C ₁₂ H ₁₃ O ₄	303 , 182 ,97	80 , 67
77	C ₆ H ₅	C ₁₁ H ₁₆ NO ₄	(105)	Not Determined
68	C ₄ H ₆ N	C ₁₃ H ₁₅ O ₄	(97), (96)	Not Determined
67	Not Det.	Not Det.	(96),(94),(82)	Not Determined
59	C ₂ H ₃ O	C ₁₅ H ₁₈ NO ₂	(152)	Not Determined
55	C ₃ H ₅ N	C ₁₄ H ₁₆ O ₄	(166),(97),(82)	Not Determined
51	(C ₄ H ₃)	-	(105), (77)	Not Determined
42	C ₂ H ₄ N	C ₁₅ H ₁₇ O ₄	(122),(108),(96)	Not Determined

^a**Boldface** indicates the most prominent precursor or product ion(s) of the group.

^bValues in parentheses are proposals based on other information, most often data from product ion scans.

^cValues in parentheses are proposals based on other information, often data from precursor ion scans.

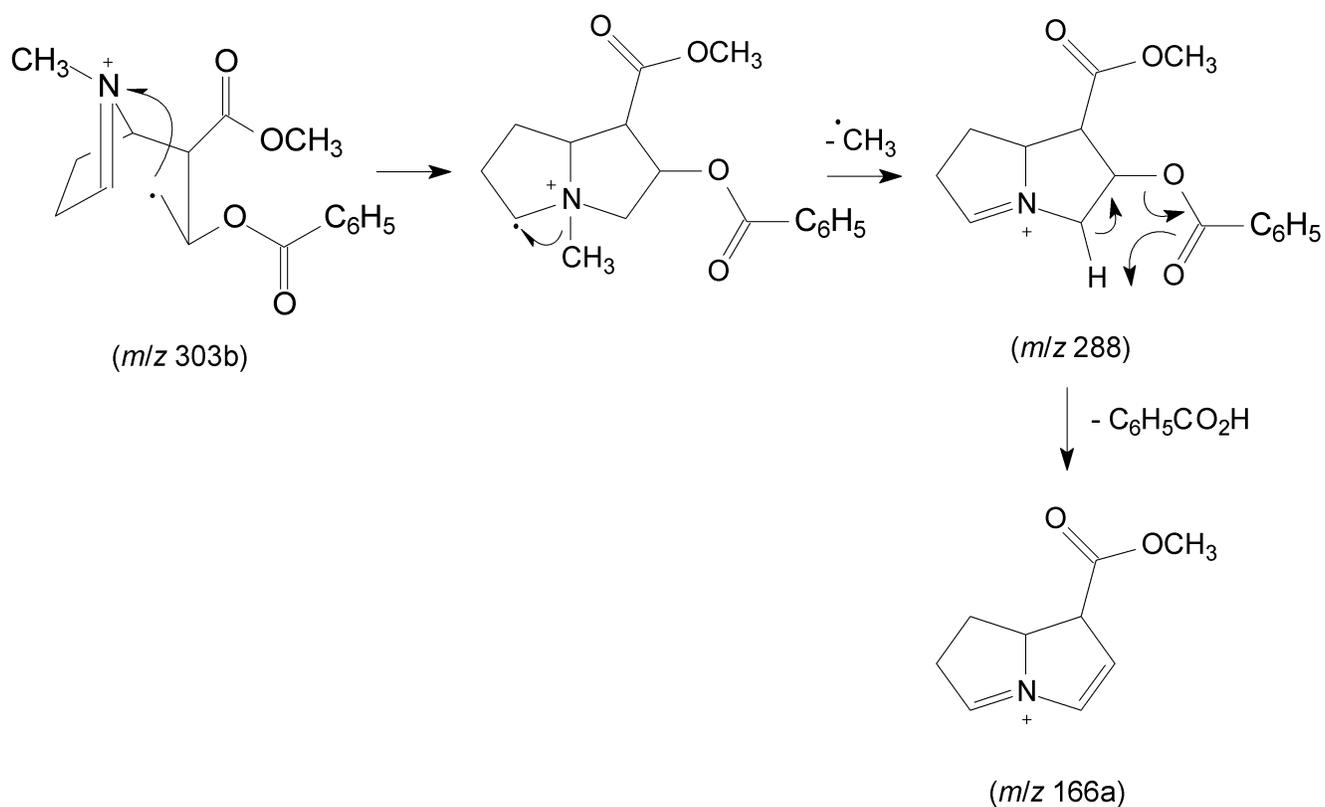


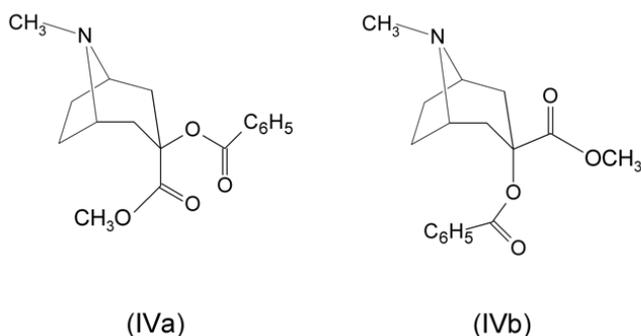
Figure 8 - Proposed formation of m/z 288 and m/z 166 from m/z 303b.

Table III. Tabulated Product Ion Spectrum of the Molecular Ion of **Ia** at Various Collision Energies.

Product Ion (m/z)	Rel. Int. (-20 eV)	Rel. Int. (-13 eV)	Rel. Int. (-5 eV)
272	-	0.5	2.0
222	-	-	5.0
198	16.0	32.0	40.0
182	4.0	3.0	2.0
181	4.0	10.0	50.0
180	1.0	-	-
166	2.0	1.0	-
122	3.0	2.0	2.0
108	5.0	4.0	2.0
107	0.5	1.0	-
100	1.0	2.0	-
97	1.0	1.0	2.0
96	1.0	0.5	-
95	1.0	2.0	5.0
94	1.0	1.0	-
83	38.0	72.0	100.0
82	100.0	100.0	60.0
81	9.0	13.0	30.0

the M^+ at low internal energies (Table III). The loss (81 u) is an unusual one, considering the functional groups present in the molecule. The spectra of the D-labeled derivatives show losses of the N-CD₃ label as well as four of the six D atoms on C1, C5, C6, and C7. These losses strongly suggest that the entire 5-membered ring, consisting of the N atom and C1, C5, C6, and C7, is lost as a unit, with two H atoms being transferred back to the remaining portion of the molecule. Because peaks representing ions having similar m/z values are very prominent in the spectrum (m/z 82 and 83), loss of this portion of the M^+ as a neutral species is not unreasonable. Loss of the N atom, in addition to the even molecular mass, dictates that m/z 222 is an odd-electron ion; therefore the lost neutral species is either a molecule or a diradical.

Two fragment ions associated with m/z 222 by MS/MS are those at m/z 122 and 100. The m/z 122 ion must be $C_6H_5CO_2H^+$ because it cannot contain the N atom. The mechanisms shown in Figure 13 account for these observations.



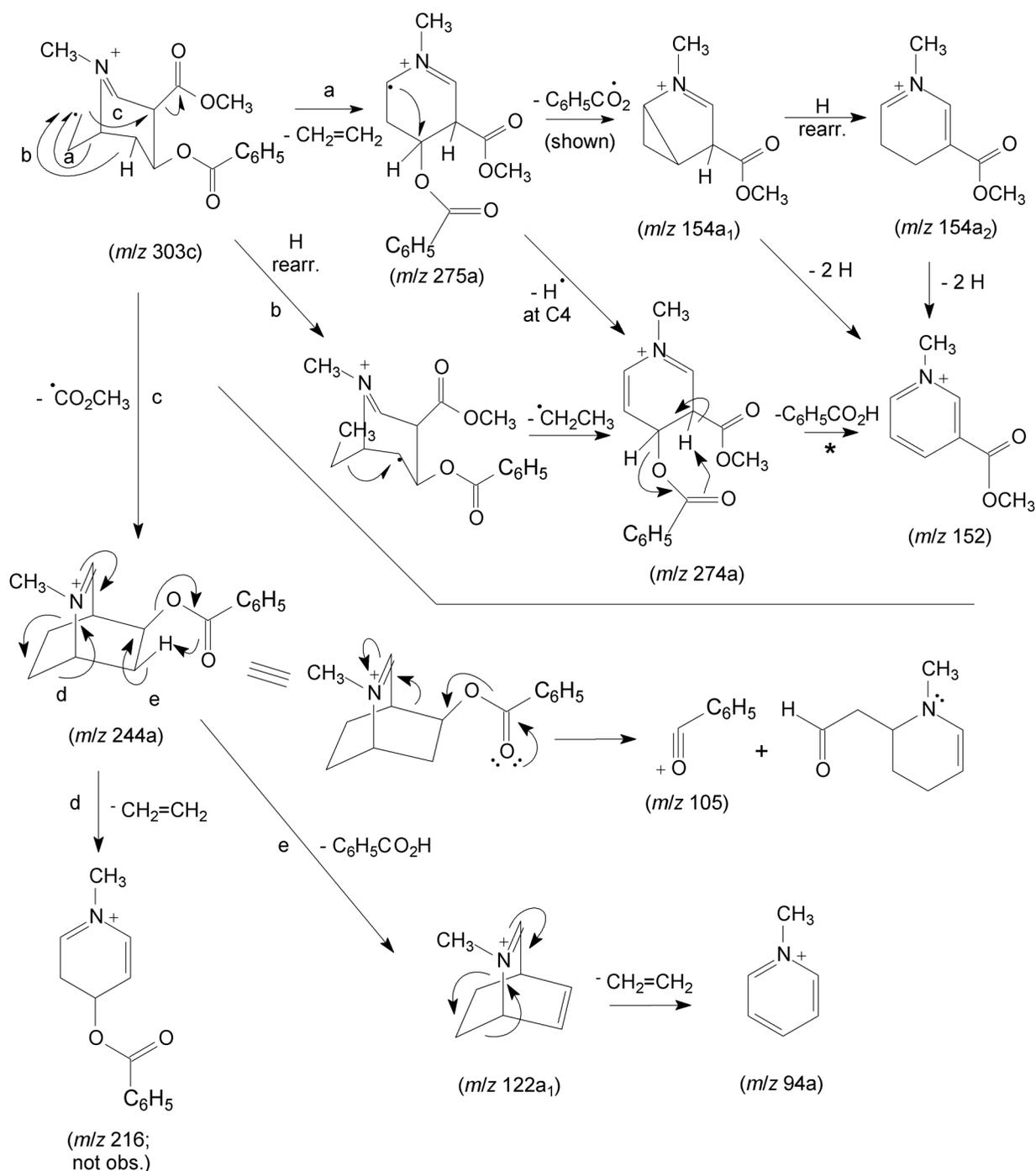


Figure 9 - Proposed formation of m/z 275a and m/z 244a after initial cleavage of the C1-C7 bond (m/z 303c). Subsequent fragmentations of m/z 275a and m/z 244a include formation of m/z 274a, m/z 216, m/z 152, m/z 122a₁, and m/z 105.

m/z 198. The combined HR-MS and MS/MS data show loss of $\text{C}_7\text{H}_5\text{O}$ directly from the M^+ (Tables II and III). The only D labels lost are those on the aromatic ring, thus the neutral fragment must be a benzoyl radical ($\text{C}_6\text{H}_5\text{CO}^\cdot$). In the spectra of α - and β -cocaine (IVa and IVb, respectively; Figure 14a – the spectra of these two compounds are virtually identical), the peak associated with this ion is quite small, indicating that formation of this ion may require the presence of a H atom at C3, which is not present in either α - or β -cocaine. The m/z 198 ion is an important precursor to one of the m/z 166 ions, which can occur by loss of a molecule of methanol (Figure 2) [4].

m/z 182. The M^+ loses $\text{C}_7\text{H}_5\text{O}_2$ – benzoate radical – to form this ion (Tables II and III). None of the D labels except those on the phenyl ring are lost. A straightforward mechanism for its formation is shown in Figure 2 [15,16].

m/z 181. Loss of benzoic acid ($\text{C}_6\text{H}_5\text{CO}_2\text{H}$) directly from the M^+ produces this ion (Tables II and III). The D labels on the aromatic ring are clearly lost in this fragmentation, and loss of a H atom from either C2 or C4 is supported. The m/z 181 ion is implicated as an intermediate in forming ions having m/z 166, 152, 122a (it cannot be $\text{C}_6\text{H}_5\text{CO}_2\text{H}^+$) and 108. Generation of m/z 181 involves a McLafferty-type rearrangement after initial

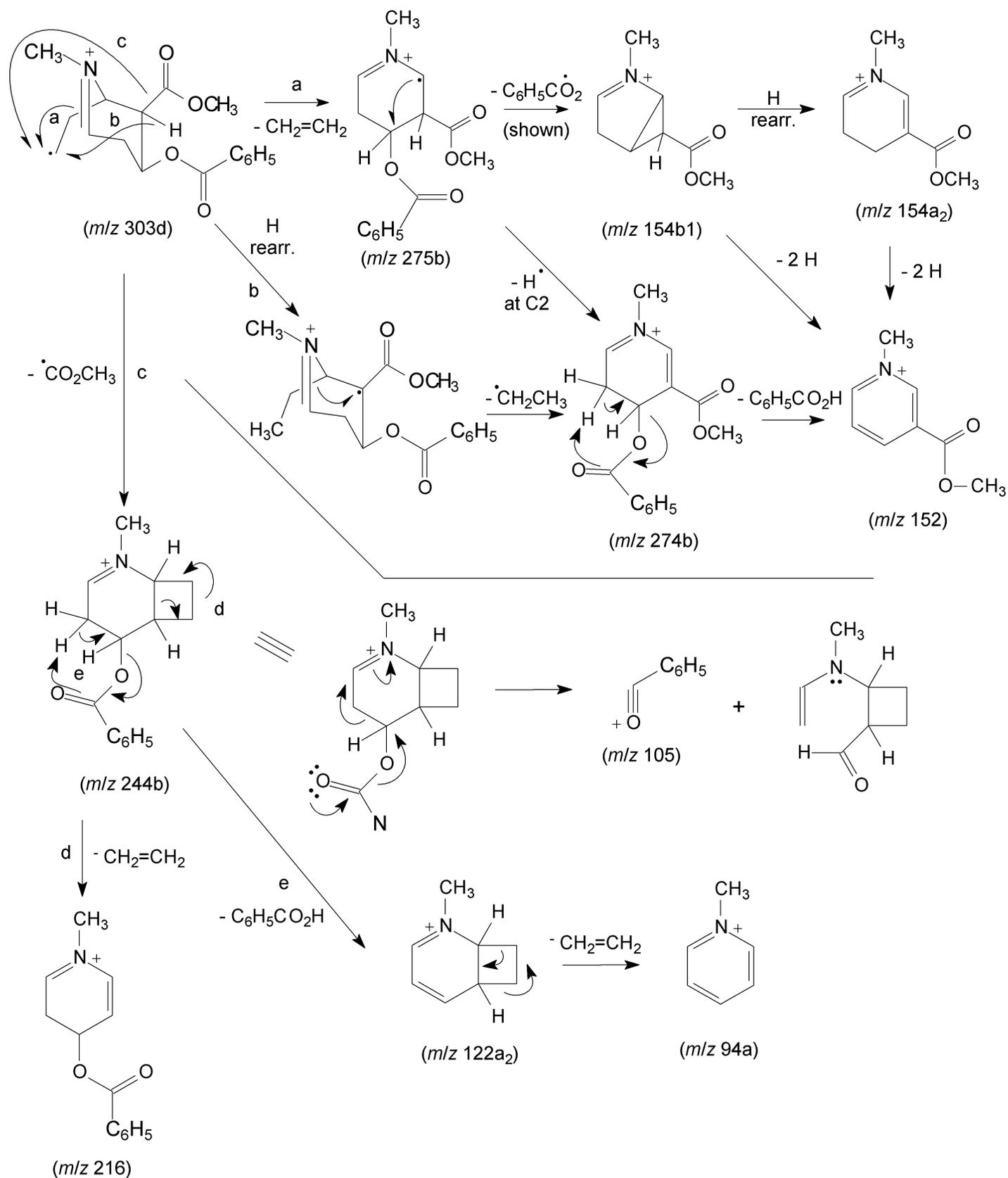


Figure 10 - Proposed formation of m/z 275b and m/z 244b after initial cleavage of the C5-C6 bond (m/z 303d). Subsequent fragmentations of m/z 275b and m/z 244b include formation of m/z 274b, m/z 216, m/z 152, m/z 122a₂, and m/z 105.

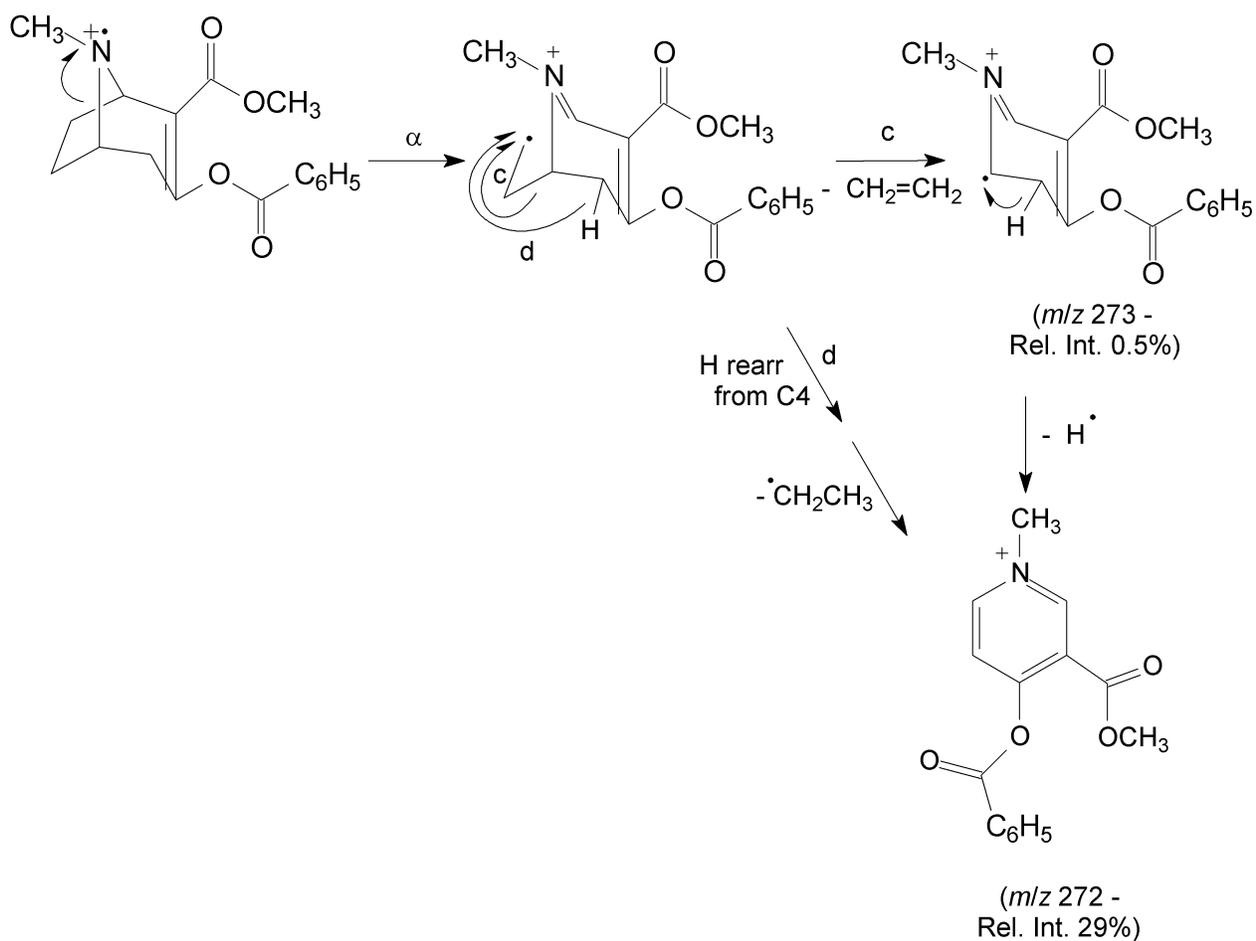
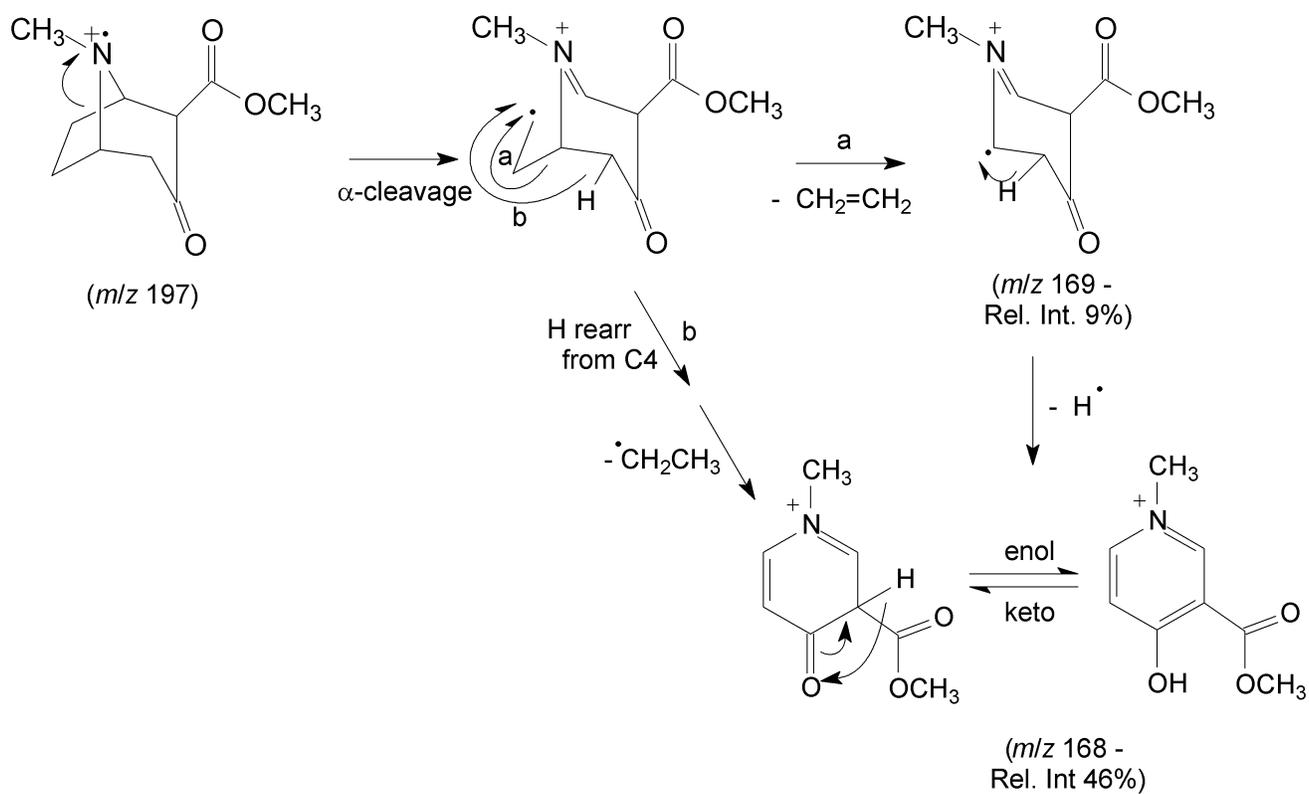


Figure 11 - Losses of C_2H_5 from 2-carbomethoxy-3-tropanone (II) and 2,3-anhydrococaine (III) produce ions stabilized by aromatic conjugation.

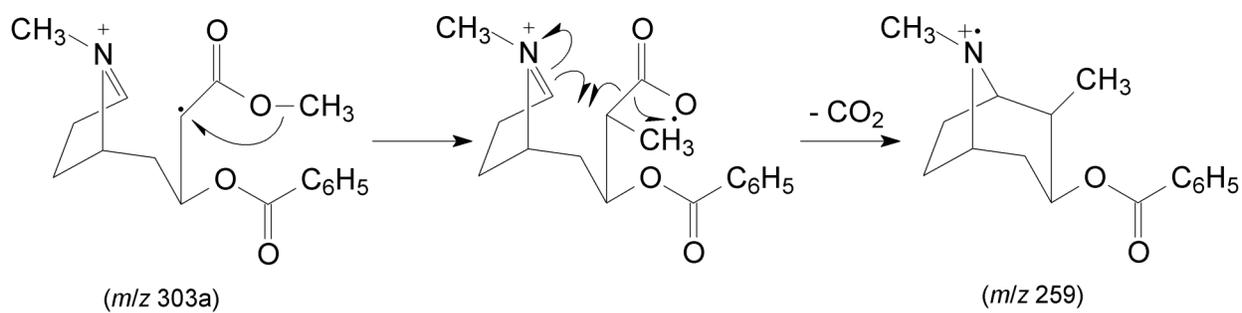


Figure 12 - Proposed formation of the m/z 259 ion.

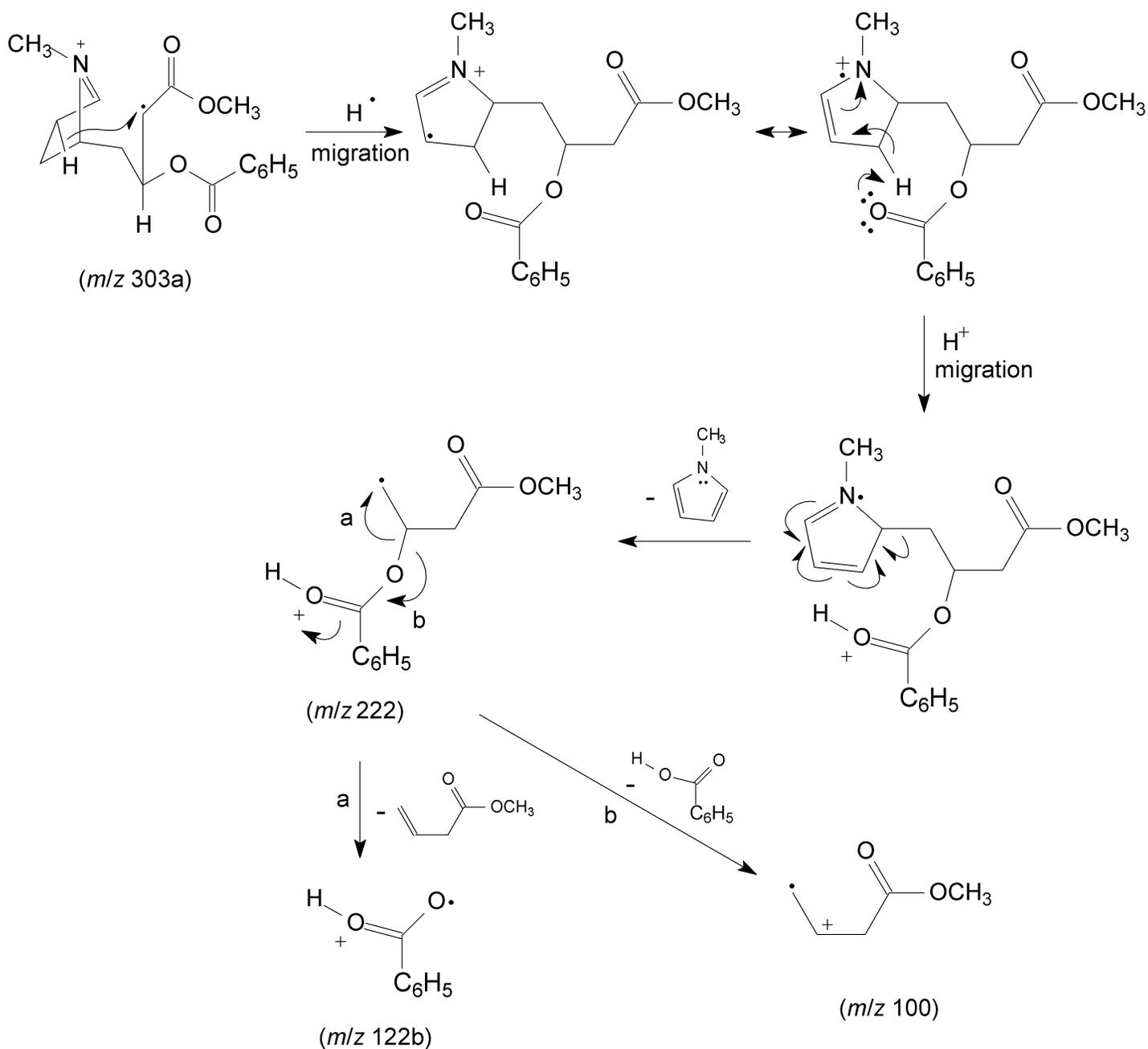


Figure 13 - Proposed formation of m/z 222 and its subsequent fragmentation to give the m/z 122 and m/z 100 ions.

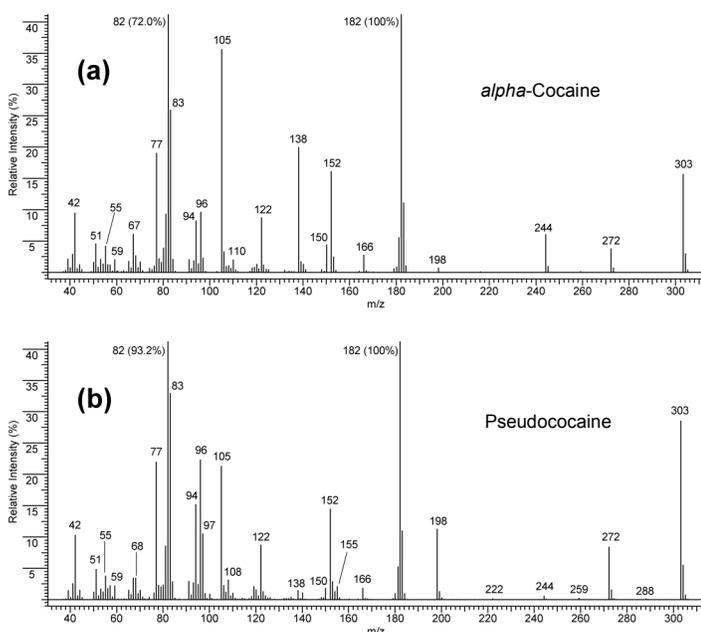
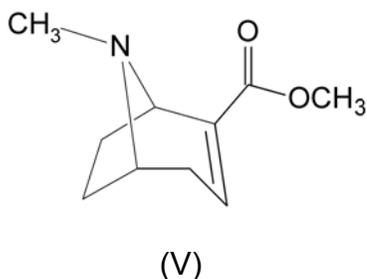


Figure 14 - Electron ionization mass spectra of a) *alpha*-cocaine (**IVa**); and b) pseudococaine.

ionization at the carbonyl O atom of the benzyloxy group [5]. In this case, the charge ends up on the olefin product. Two isomeric structures for m/z 181 are possible; they appear to suffer different fates (Figures 15 and 16).

m/z 166. Determining the origin of the m/z 166 peak illustrates the danger of assuming there is a one-to-one correlation between ions and peaks in a low-resolution EI mass spectrum. The MS/MS data indicates that the m/z 166 ions might have at least three precursor ions – particularly the ones having m/z 288, 198, and 181. Formation of m/z 166 from m/z 288 and 181 involves loss of benzoic acid and a methyl radical, whereas production of this ion from m/z 198 involves loss of benzoyl radical and a molecule of methanol. In each case, the total loss is $C_8H_9O_2$, consistent with the elemental composition (Table II). As can be seen from the previously proposed structures for the precursor ions, it seems likely that there are at least three isomeric m/z 166 ions.

The D labeling data is understandably complex. Although the labels on the aromatic ring are lost, none of the other derivatives show complete loss or retention of individual labels. Both the OCD_3 and NCD_3 derivatives show partial loss of their respective labels, with somewhat more OCD_3 label lost than NCD_3 . Fragmentation of D-labeled 2,3-anhydroecgonine methyl ester (methylecgonidine; **V**), whose M^+ isoelectronic with m/z 181b, shows similar behavior [17]. This means that the methyl radical is lost to some extent from the N atom, and



the methyl group in the ester can be lost as either a methyl radical or a molecule of methanol.

At the remaining atoms, the H atom at C3, one H atom at C4, and one H atom from C1, C5, C6, and C7, are all lost to some degree, but only the loss at C3 is notably significant. The m/z 166 ion may fragment to produce the ions having m/z 138 and 82. The individual mechanisms shown in Figures 2 (losses at C3 and O- CH_3), 8 (losses at C2, C4, and N- CH_3), and 17 (losses at C4 and O- CH_3 or N- CH_3) explain various aspects of the data [18]. Still other mechanisms are possible.

m/z 155. Although precursor ion spectra for this ion were not recorded, its most likely source is M^+ because it can be formed in a single step from m/z 303b (Figure 18) [1,2,16]. Both D labels are lost from C4, as are those from the aromatic ring and from C3, indicating that C3, C4, and the functional groups attached to C3 are lost as a single unit. This is consistent with the size of the lost neutral fragment ($C_9H_8O_2 = C_6H_5CO_2 + CH_2=CH$). The m/z 155 peak is absent from the spectra of both α - and β -cocaine because in these compounds both the benzoate and carbomethoxy groups are located on C3, necessitating the loss of both functional groups in this fragmentation. Important product ions of m/z 155 in the MS/MS experiments include m/z 140, 96, and 82. Fragmentations that might produce m/z 140 and 82 are shown in Figure 18.

m/z 154. The m/z 154 peak can easily be overlooked in the group of peaks between m/z 150 and 155. The elemental composition of this ion indicates loss of $C_9H_9O_2$ from the M^+ . The spectra of the D-labeled derivatives show loss of the labels on the phenyl ring, and at least four D atoms are lost from C1, C5, C6, and C7. Loss of the benzoate group ($C_7H_5O_2$) along with the C6-C7 bridge (C_2H_4) is consistent with these data.

The only precursor identified for m/z 154 by MS/MS was the m/z 155 ion; however, the structure for this ion must be different from the one indicated by the D labeling data because, as just discussed, m/z 155 no longer contains C3 and C4, rather than C6 and C7 (Figure 18). A more reasonable precursor for the ion represented in the EI spectrum is one having m/z 275, which is not represented in the spectrum but is a realistic intermediate to the m/z 274 ion (Figures 9 and 10).

From the MS/MS data, it appears that the m/z 154 ion may fragment to give m/z 152, 122, and 94. The structure of the m/z 122 ion that is formed under these conditions is not clear, but it seems certain that it is neither m/z 122a nor 122b because the formal loss of CH_3OH is indicated (thus the resultant m/z 122 ion must contain an O atom) and the benzoate group was already lost in forming m/z 154. Although a third m/z 122 ion containing both N and O is represented in the spectra of the other cocaine diastereomers at low abundance, it is not observed in the cocaine spectrum.

m/z 152. The elemental composition for this ion indicates the loss of $C_9H_{11}O_2$ from the M^+ . Both the N- CD_3 and O- CD_3 labels are retained, but the phenyl label is lost. Losses of D from the tropane skeleton are complex, but losses of D at C2, one D atom at C4 and four D atoms from the C1, C5, C6, and C7 (consistent with loss of the C6-C7 bridge) are supported. Other D losses occur, however, indicating that formation of this ion must follow more than one pathway. These data, along

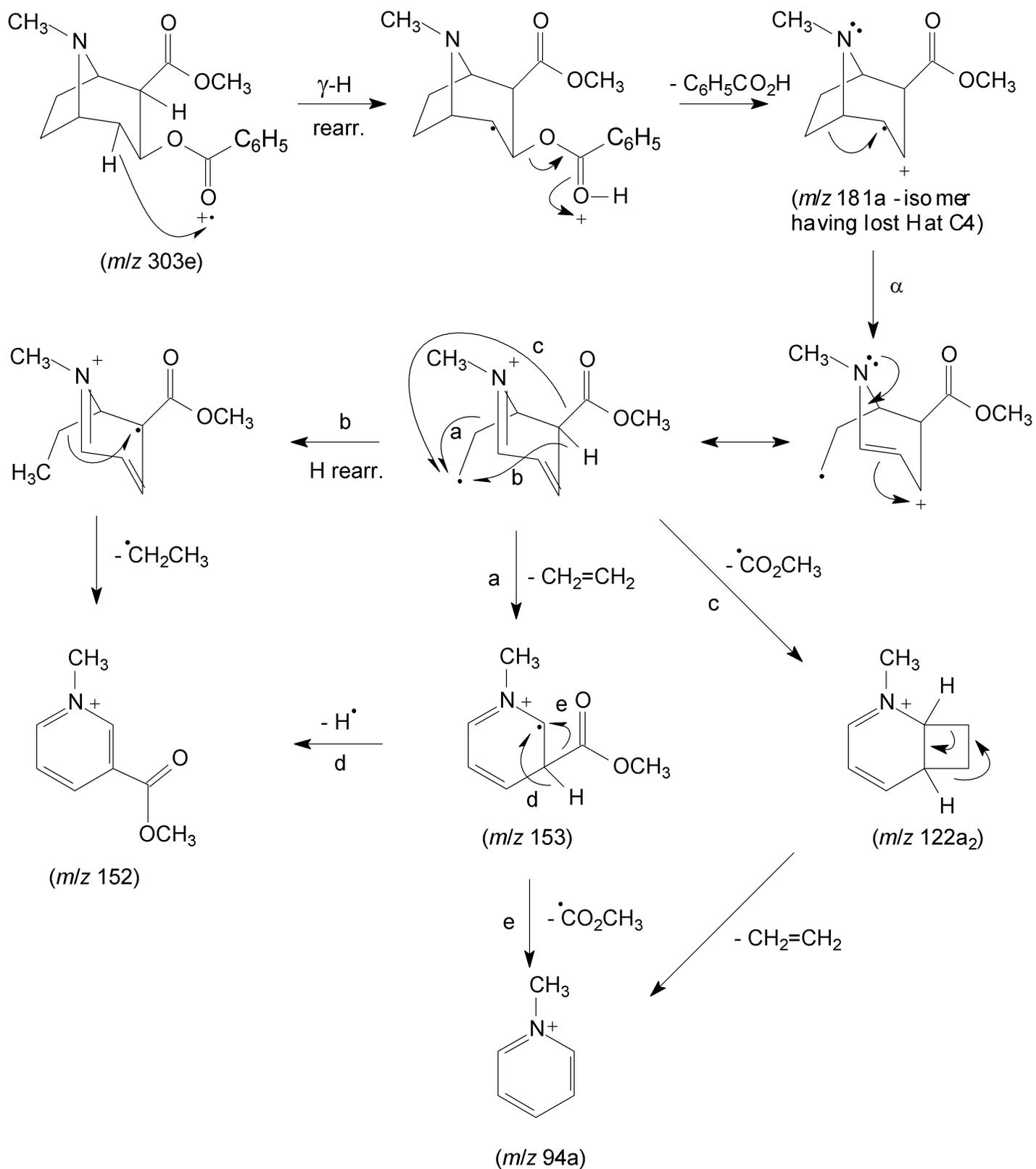


Figure 15 - Proposed formation of m/z 181a (by loss of the H atom at C4) and its subsequent fragmentations.

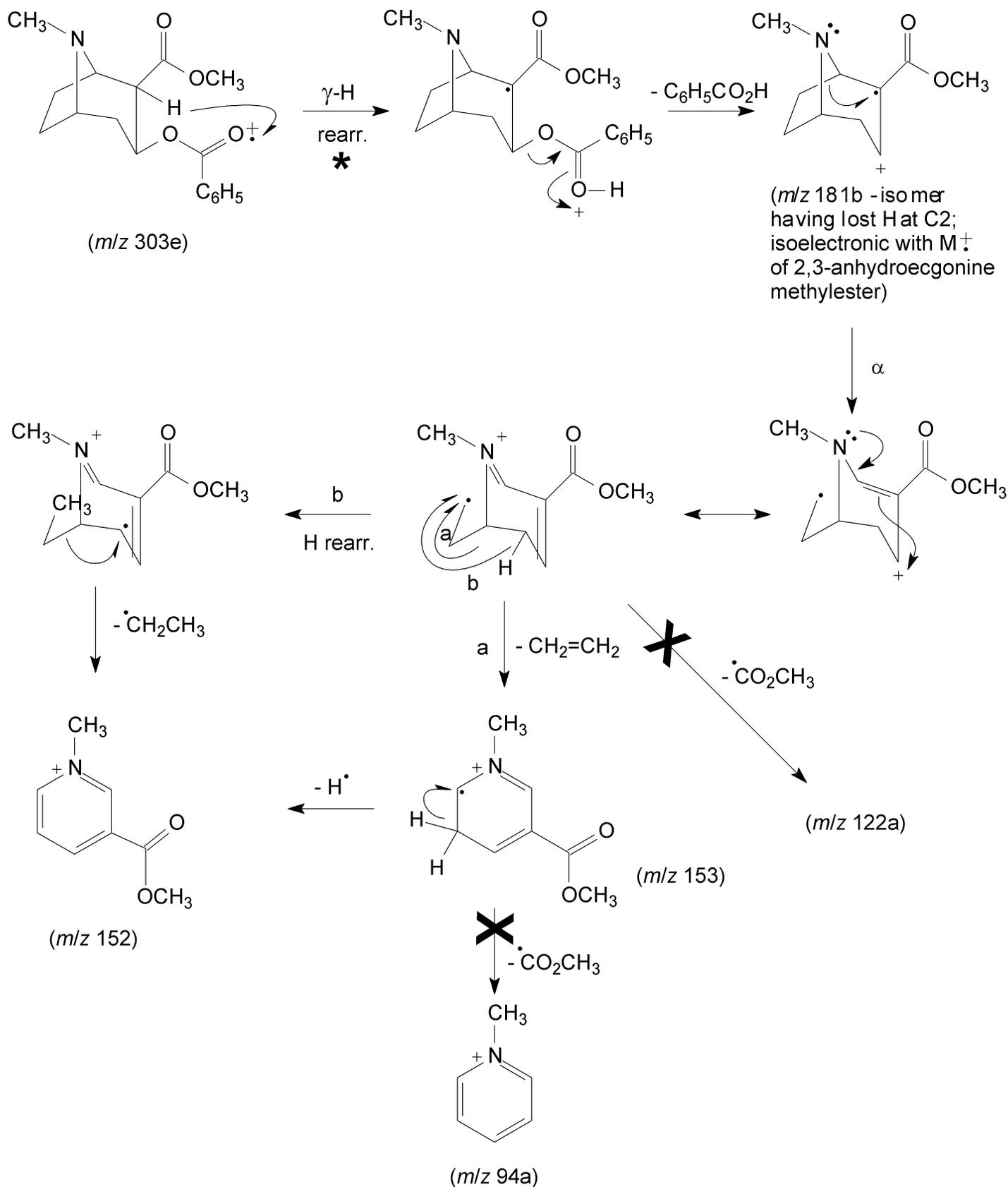


Figure 16 - Proposed formation of m/z 181b (by loss of the H atom at C2) and its subsequent fragmentations.

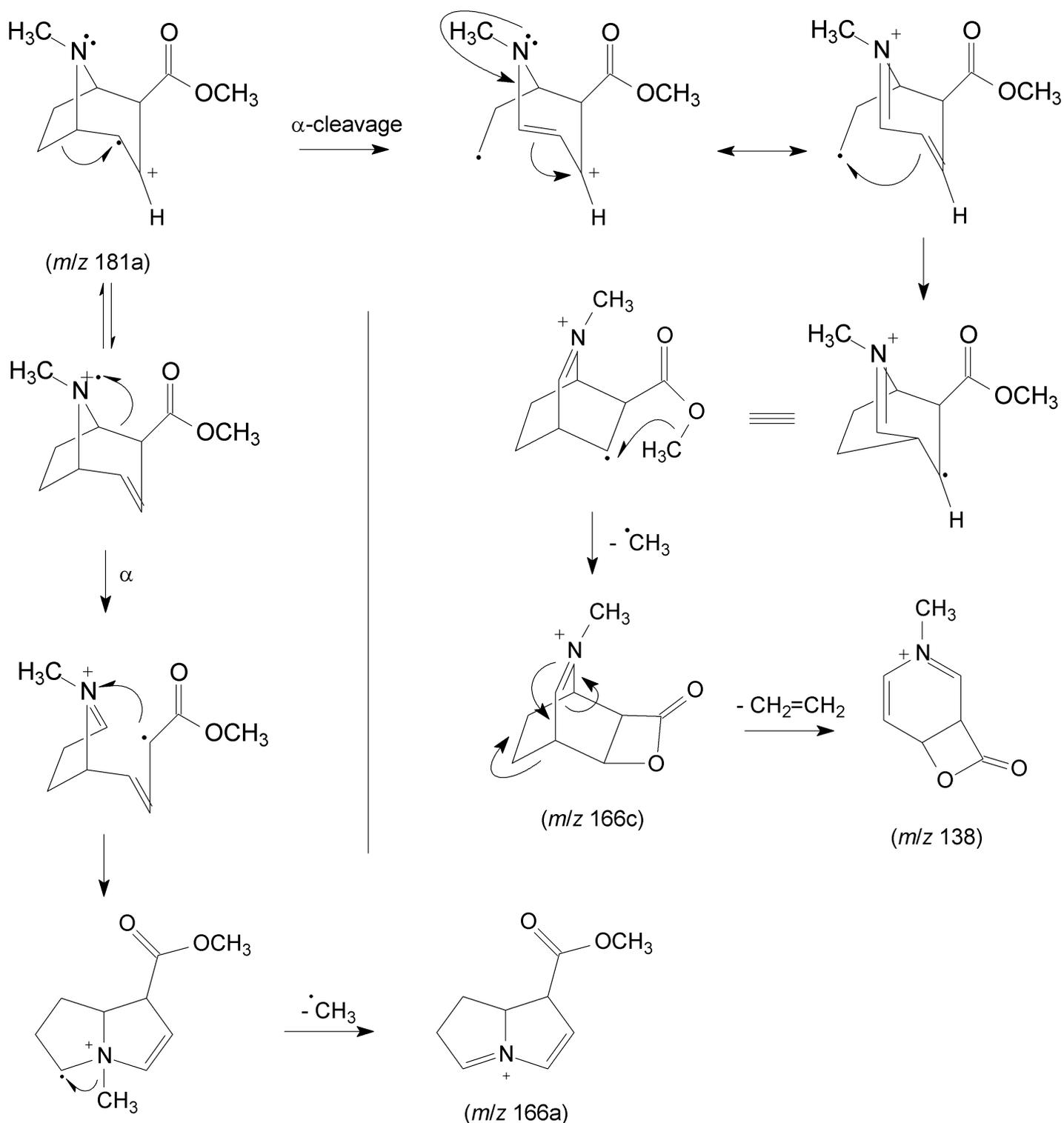


Figure 17 - Possible mechanisms for formation of m/z 166a and m/z 166c from m/z 181a.

with the elemental composition, primarily indicate formal loss of a molecule of benzoic acid plus an ethyl radical. The most likely structure is the aromatic 3-carbomethoxy-N-methylpyridinium ion [5,13,15,19].

Although some m/z 152 ions result from fragmentation of m/z 154 at very low collision energies (Table II), two more important precursors appear to be the m/z 274 and 181 ions. At collision energies of 6-20 eV, both are significant contributors.

Both these ions are reasonable intermediates for the EI fragmentations leading to m/z 152. Formation of m/z 152 via m/z 274 involves α -cleavage from the M^+ with subsequent loss of the C6-C7 bridge along with a H atom from either C4 or C2, followed by cyclic loss of a molecule of benzoic acid involving a H atom at whichever C atom (C2 or C4) is still saturated (Figures 9 and 10). When m/z 152 is formed via m/z 181, this process is formally reversed (Figures 15 and 16). Although one

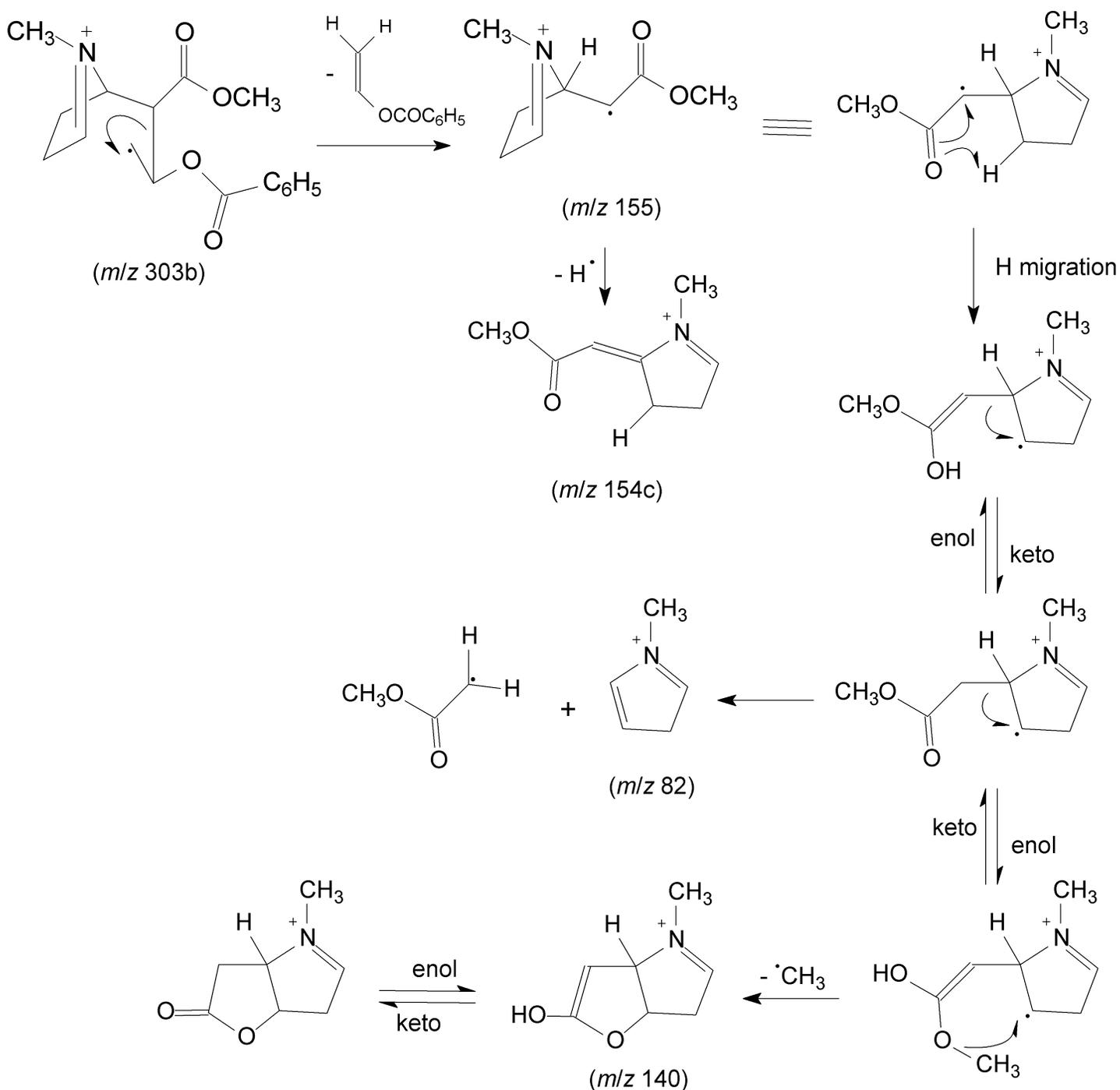


Figure 18 - Proposed formation of m/z 155 and its subsequent fragmentations.

might assume that the double bond in either process would form initially between C2 and C3 because it would be conjugated with the double bond in the carbonyl group at C2, there is no clear evidence that this is the case.

The intensity of the m/z 152 peak for cocaine (0.4% of total peak intensities; Table IV) is significantly smaller than it is in the spectra of pseudococaine (Figure 14b), allococaine, and α - and β -cocaine (approximately 2%). The intensity of m/z 152 in the spectrum of pseudoallococaine has an intermediate value. The relative size of this peak is often used to distinguish the spectrum of cocaine from those of the other diastereomers [3,5,7,9,20]. Trying to explain why this is so has been a goal of forensic chemists for many years.

Both of the fragmentation pathways to m/z 152 proposed here involve steps that are potentially sensitive to the relative stereochemistry at C2 and C3. First, loss of the C6-C7 bridge as an ethyl radical involves a 5-center H rearrangement from either C2 or C4 to the radical site at C6 or C7 (Figures 9, 10, 15, and 16). Although this rearrangement might be influenced by the relative position of the migrating H atom, it is clear that when the H atom is initially *endo* to the ring system the rearrangement occurs with consummate facility; in the spectrum of 2,3-anhydroecgonine methyl ester (V), this fragmentation accounts for the base peak in the spectrum (Figure 16). Even when the migrating H atom is initially *exo* to the ring system and becomes *trans* to the C6-C7 bridge, molecular models

indicate that it can be approached by the radical site nearly as well as when the H atom is *cis* to the bridge.

On the other hand, cyclic loss of benzoic acid – whether from the M^+ via the McLafferty (γ -hydrogen) rearrangement or from the m/z 274 ion by a superficially similar type of rearrangement – should proceed readily only when the carbonyl O atom and the migrating H atom can approach one another easily. This can occur by migration of a H atom from either C2 or C4, depending upon which H atoms are available (Figures 9, 10, 15, and 16). Access to an available H atom at C4 should be equally facile for all of the cocaine diastereomers in both fragmentation schemes, since one of the two available H atoms at C4 will necessarily be “*cis*” to the benzoate group (Figures 10 and 16). At C2, the benzoate group and single available H atom are “*cis*” to one another only in pseudo- and allococaine (Figure 19; also Figure 16, indicated by *; and Table IV).

When m/z 152 is formed via m/z 274, the six-membered ring in the m/z 274 ion has two π -bonds that force rigidity (Figure 20, indicated by *). Again the 2-H and 3-benzoyloxy groups are *cis* to one another in pseudo- and allococaine, but *trans* to one another in cocaine and pseudoallococaine. With α - and β -cocaine, in which both functional groups are located on C3, a choice of migrating H atoms is always offered at both C2 and C4, so that stereochemical relationships are not an issue, and a more intense m/z 152 peak is observed.

This rationalization does not explain the subtle differences that are observed – especially that the m/z 152 peak is larger in the pseudoallococaine spectrum than in that of cocaine. However, it should be apparent that because more than one pathway exists for formation of m/z 152, and because of the probable difference in conformation of the ring between the 3-*exo* and 3-*endo* isomers (Figure 19), a more nuanced analysis is not possible on the basis of the data presented here.

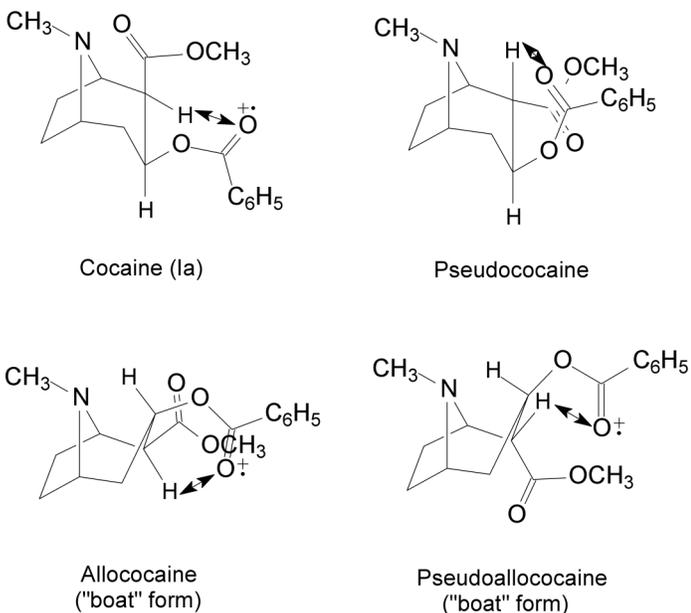


Figure 19 - Schematic representations of stereochemical relationships present in the loss of benzoic acid from the M^+ in the four cocaine diastereomers.

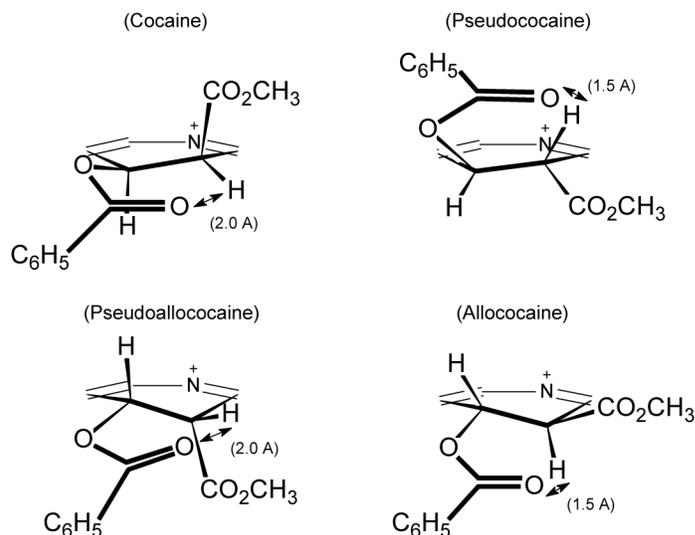


Figure 20 - Schematic representations of stereochemical relationships present in the loss of benzoic acid from m/z 274 in the four cocaine diastereomers.

m/z 150. Two ions give the m/z 150 ion as a significant product ion in the MS/MS experiments – those having m/z 272 and 182. The first instance involves overall loss of a methoxy radical plus a molecule of benzoic acid; the latter, loss of a benzoate radical and a molecule of methanol. Loss of both the OCD_3 and phenyl labels in this ion is consistent with this interpretation. The D labels in the tropane skeleton are difficult to interpret because of interference by other peaks in this region of the spectrum. Nonetheless, it appears that there may be loss of D from C4 or from the C1, C5, C6, and C7 portion of the molecule. At low collision energies, m/z 150 loses CO to produce m/z 122a.

Mechanisms that are consistent with these data are shown in Figures 3 and 21, although additional ones are possible. Both mechanisms show the H atom involved in the cyclic losses of benzoic acid and methanol coming from C4. The two-dimensional diagrams in Figure 21 do not adequately show that the methoxy O atom and the H atom on C4 can approach each other as close as about 1.5 Å.

m/z 140. This ion appears to be formed by loss of a methyl radical from the m/z 155 ion (Table II). The pattern of D loss from the tropane skeleton is the same as that for m/z 155, but the additional loss of the O-methyl group is seen. Figure 18 depicts a mechanism that is consistent with this observation.

m/z 138. The only precursor identified for m/z 138 in the MS/MS experiments is an ion having m/z 166, but it is not clear which one. From the elemental composition, it is apparent that the lost fragment is $CH_2=CH_2$, not CO. Both the methyl ester and phenyl groups are lost, but the N-methyl group is retained. Losses from the tropane skeleton are harder to discern. Although the D atoms at C2 and C3 appear to be retained, three or four D atoms are lost from the d_6 derivative. This could indicate loss of the C6-C7 bridge. A simple mechanism involving a reverse cycloaddition fragmentation from m/z 166c is consistent with these data (Figure 17).

Table IV - Stereochemical Constraints to Rearrangement at C2 (m/z 152).

Diastereomer	Intensity of m/z 152 ^a	C2 H to Benzoyl O Distance (Å) ^b	
		Via m/z 274	Via m/z 181
Cocaine	0.4%	2.0	2.0 (chair)
Pseudococaine	1.6%	1.5	1.6 (chair)
Allococaine	2.1%	1.5	1.4 (boat)
Pseudoallococaine	0.7%	2.0	1.8 (boat)

^aAs percentage of sum of intensities measured for all peaks between m/z 35 and 310. Ref. 5.

^bApproximate interatomic distances determined from framework molecular models.

m/z 122. There are two ions having different elemental compositions recorded at m/z 122. High-resolution MS shows that one of these ions, designated as m/z 122a, has the formula $C_8H_{12}N$. The second (m/z 122b) is the benzoic acid radical ion. It is important to remember that the MS/MS studies cannot distinguish between these ions.

(a) In precursor MS/MS spectra, both m/z 181 and 150 produce m/z 122a. Mass-analyzed ion kinetic energy studies done by Shapiro, *et al.* also identified the m/z 181 ion as precursor [9]. These fragmentations involve loss of 59 u (the carbomethoxy group) from m/z 181 and CO from m/z 150.

The D labeling data must be analyzed with caution because of the presence of the two m/z 122 ions. Both our own (0.8% vs. 5.8%) and Shapiro's HR-MS work [9] show that m/z 122a is significantly more abundant than m/z 122b. This is also reflected in the spectra of the D-labeled derivatives **1c** and **1d**, in that the m/z 125 peak (reflecting the presence of the N- CD_3 label) is much more intense than m/z 122 in Figure 4b, but m/z 127 is only a small peak in Figure 6b (reflecting retention of the phenyl D labels). Therefore, most of the observable shifts in the spectra of the deuterated derivatives are due to m/z 122a, not m/z 122b.

Although the methyl ester and aromatic ring are absent in m/z 122a and the N-methyl group is retained, the remaining D labeling data are complex. The other derivatives show a pattern in which the D atoms at C2 are lost about 40% of the time; 25% of those at C3 are lost; and two D atoms are lost from C4 about 30% of the time, but only one D is lost about another 30% of the time. The d_6 -derivative exhibits retention of all six D atoms (35%), loss of one D (35%), loss of five D (15%), and loss of all 6 D (15%). In this case, the losses of 5-6 D atoms are more logically associated with the m/z 122b ion or perhaps even with the m/z 119 ion, indicating that the C6-C7 bridge is probably retained in m/z 122a.

One cannot reasonably postulate a single, simple mechanism that accounts for this pattern of losses. Figures 9, 10, 15, and 21 show several possible mechanisms that together are consistent with the predominant retention of H atoms at all skeletal positions except C2 and C4 [16]. Note that m/z 122a cannot be formed easily from m/z 181b (Figure 16).

(b) Because of the elemental composition and presence of the aromatic ring, the m/z 122b ion is assigned the benzoic acid radical ion structure. An important precursor appears to be the M^+ , although other ions undoubtedly also play a role (Table II). Initial ionization at the benzoate group, followed by McLafferty

rearrangement involving removal of a H atom either from C2 or C4, leads to m/z 122b (Figure 3). Fragmentation of the m/z 122b ion leads to the benzoyl ion (m/z 105) and its known fragment ions (m/z 77 and 51) [21]. The patterns of D losses observed for m/z 105, 77, and 51 are all consistent with this assignment.

m/z 108. Taken as a whole, the data for the m/z 108 ion contain a contradiction that cannot be resolved without invoking two separate mechanisms. The N- CH_3 group is clearly retained because of the labeling and elemental composition data, but both ester groups are lost. On the tropane skeleton most of the D label at C2 is lost, most of the label at C3 is retained, and about 50% of one label at C4 is lost, as are one or two D atoms from C1, C5, C6, and C7. This strongly indicates that *the C6-C7 bridge remains intact* in this ion and implies that the seven C atoms in the ion consist of the N-methyl group plus all the atoms in the tropane skeleton except C2. However, the MS/MS data show that the ions having m/z 182, 181, and 152 can all act as precursors to m/z 108. In the last case, *the C6-C7 bridge is no longer present*. This indicates that the m/z 108 ion is formed from different precursors under EIMS and MS/MS conditions.

Formation of m/z 108 from m/z 152 involves the loss of a molecule of CO_2 , leading to a stable aromatic ion (Table II and Figure 22a). However, production of m/z 108 from either m/z 181 or 182 seems to involve loss of the carbomethoxy group, C2, and a H atom from C5 (Figure 22b). Other fragmentation schemes, as well as other structures for m/z 108, are possible.

m/z 105. See discussion under m/z 122b above and Figure 3.

m/z 100. The m/z 222 ion appears to fragment to m/z 122b and m/z 100 at lower collision energies. Analysis of the spectra of the D-labeled derivatives for the m/z 100 ion shows retention of the O- CD_3 group, as well as the D atoms at C2, C3, and one D atom at C4. The N- CD_3 and the phenyl groups are lost. Although the pattern of losses in the d_6 -derivative could not be determined with certainty, loss of the five-membered ring consisting of C1, the N atom, C5, C6, and C7 is likely simply because the retained mass indicated by the apparent presence of the carbomethoxy group, C2, C3, and C4 is sufficient. A mechanism for formation of this ion that both accounts for these data and is consistent with the elemental composition in Table II is shown in Figure 13.

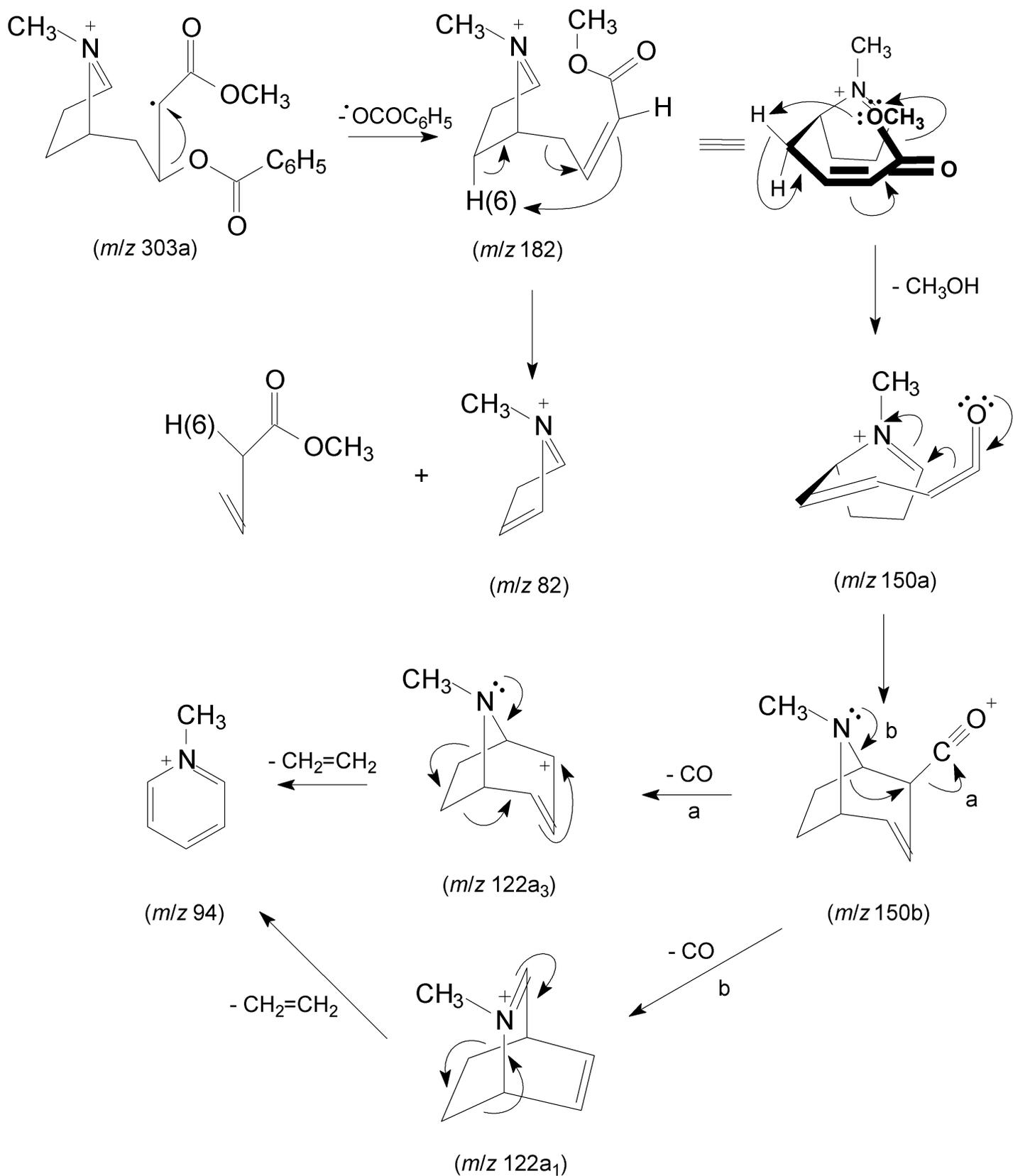


Figure 21 - Proposed formation of m/z 150, m/z 122, m/z 94, and m/z 82 from m/z 182.

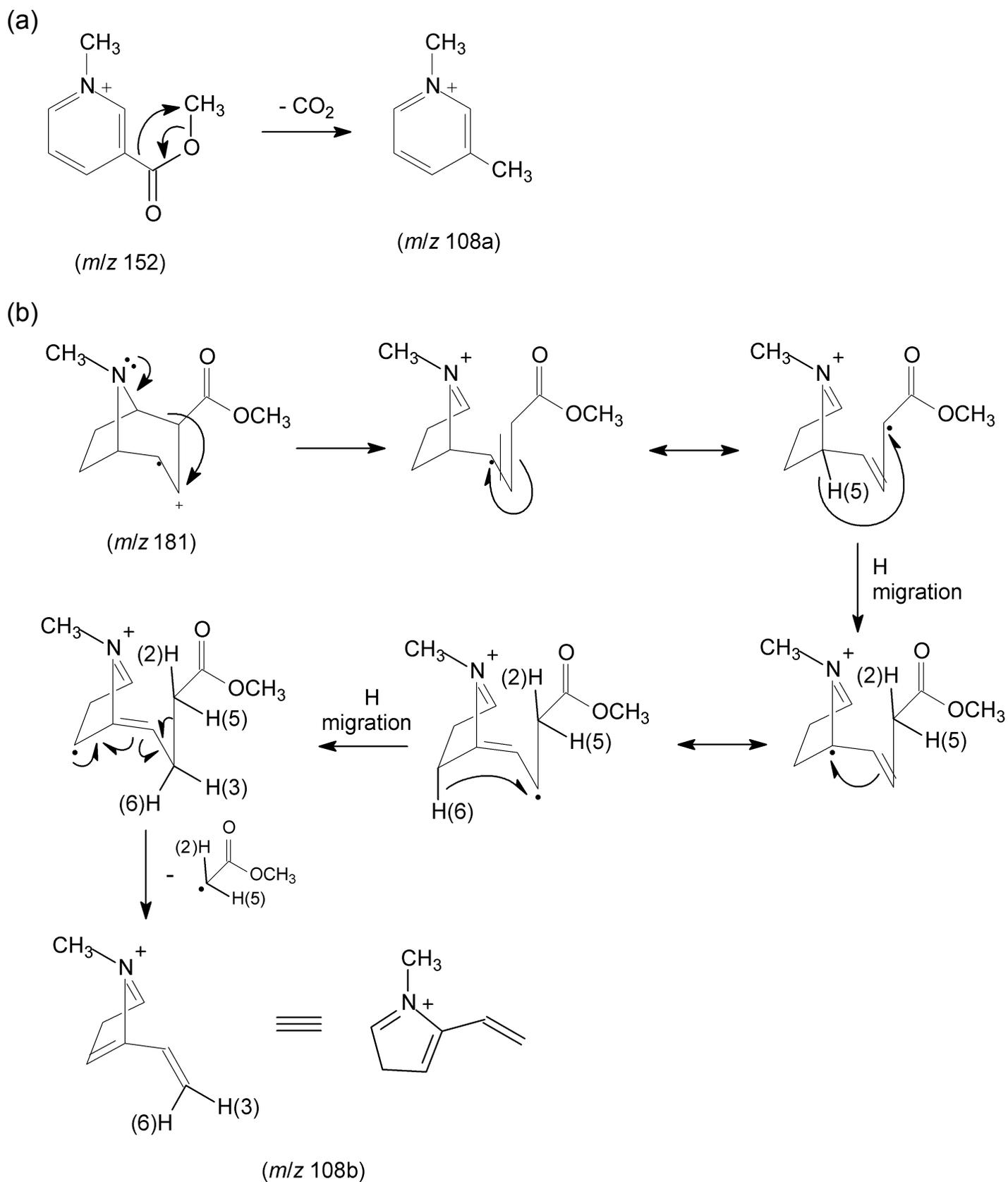


Figure 22 - Possible mechanisms for formation of *m/z* 108 from a) *m/z* 152; and b) *m/z* 181.

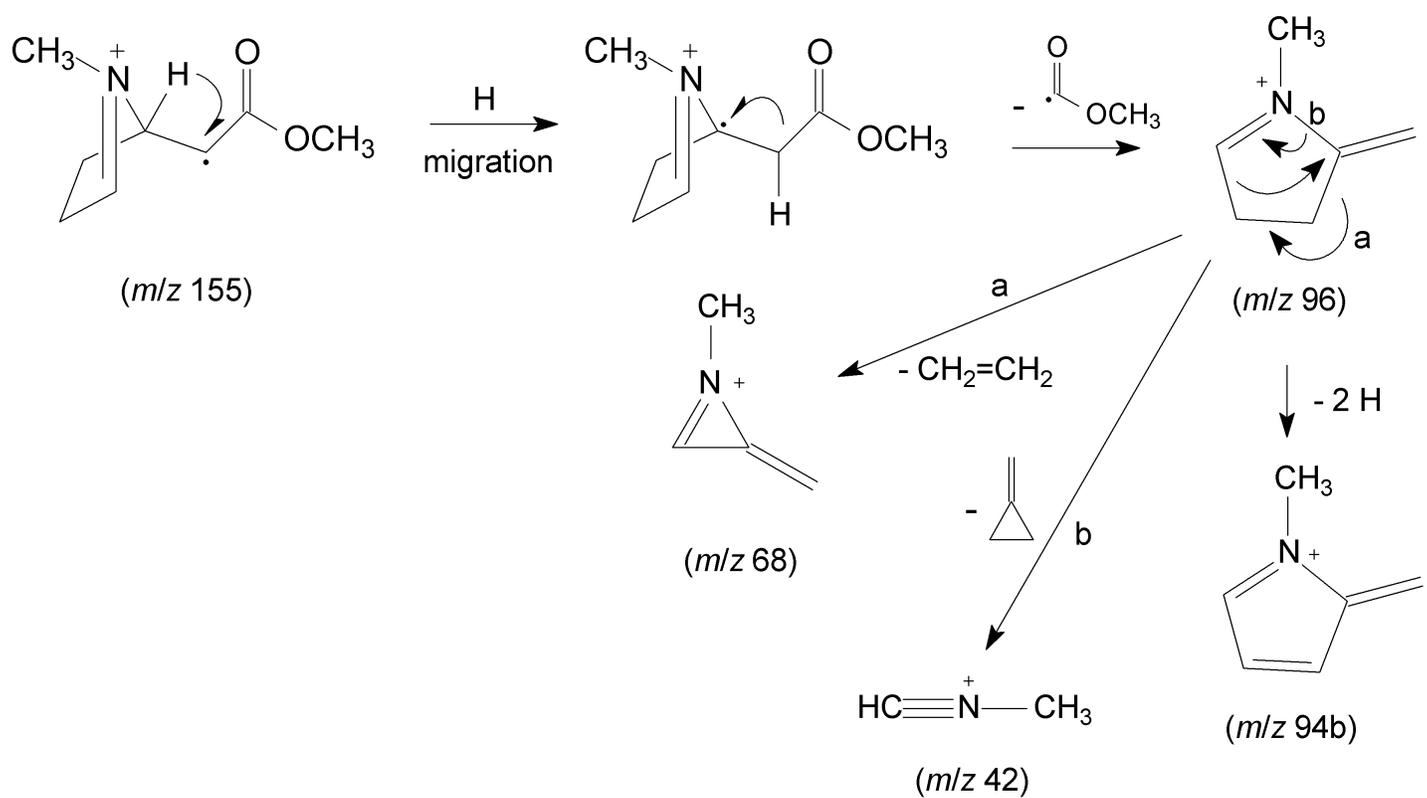


Figure 23 - Proposed formation of *m/z* 96 and its fragments from *m/z* 155.

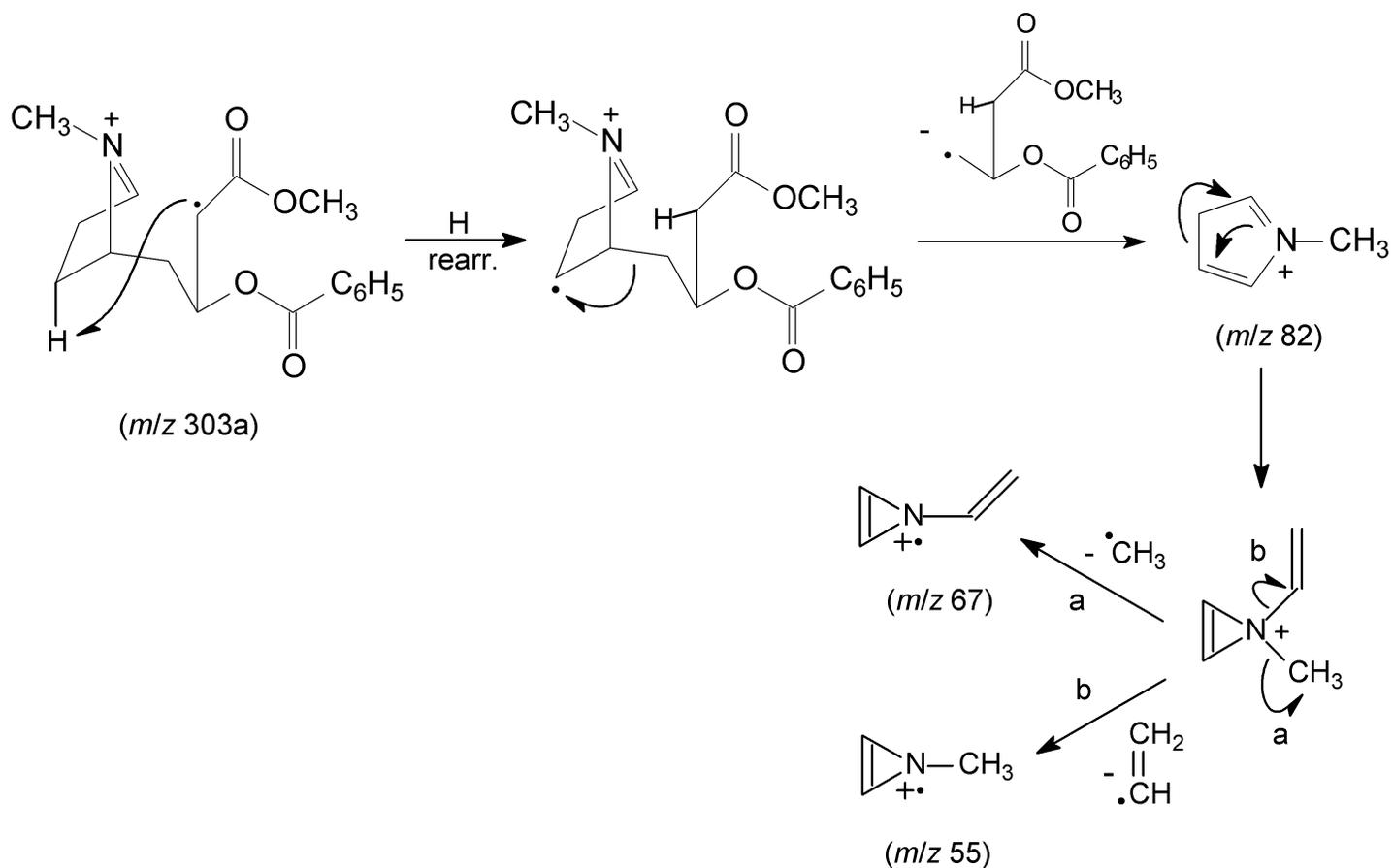


Figure 24 - Proposed fragmentations of *m/z* 82 to give *m/z* 67 and *m/z* 55.

***m/z* 97.** The principal precursor to this ion is the M^+ , especially at low collision energies (Table III). Deuterium loss patterns are difficult to interpret because of the proximity of other more intense peaks in this area of the spectrum. Nonetheless, it appears that the D atoms at C2 and C3, as well as one D atom from C4, may be lost. No more than 1 D atom is lost from the remainder of the tropane skeleton, indicating that the C6-C7 bridge is retained. The N-methyl group is retained, but both ester groups are lost. A simple mechanism that explains most of these data is shown in Figure 2 [1,2,13].

***m/z* 96.** Major precursors to the *m/z* 96 ion at low collision energies are the ones having *m/z* 155 and 97. This strongly suggests that this ion, like those of its precursors, contains the five-membered ring portion of the tropane skeleton, rather than the six-membered ring. Losses from the D-labeled derivatives bear this out. Although there is loss of D at C2 and significant loss at C3, no more than one D atom is lost from C1, C5, C6, and C7, showing that the C6-C7 bridge is retained. The loss of the label from C4 could not be determined with certainty. The N-methyl group is retained, but both ester groups are lost. Two mechanisms are shown: the one in Figure 2 starts with *m/z* 97 [1,2,13]; the other (Figure 23) begins with *m/z* 155. Additional mechanisms are possible.

***m/z* 94.** In contrast to the *m/z* 96 ion, many of the *m/z* 94 ions appear to have an N-methylpyridinium structure [1,5,13,16]. The most important precursors for this ion at low collision energies are *m/z* 181 and 122a. The N-methyl group is retained, as are the H atoms at C2 and C3. Both ester groups are absent, as are one H atom from C4 and four H atoms from the C1, C5, C6, and C7 portion of the molecule. This is similar to the pattern of losses exhibited by *m/z* 152 and is consistent with loss of the C6-C7 bridge. Formation of this ion from *m/z* 122a can occur readily via a reverse cycloaddition loss of ethylene (Figures 3, 9, 10, 15, and 21). All reasonable pathways to *m/z* 94 from *m/z* 181 appear to utilize *m/z* 122a as an intermediate.

An undetermined percentage of *m/z* 94 ions have *m/z* 96 as their precursor, indicating that they probably have a structure in which the five-membered ring is intact (Figure 2).

Like the intensity of the *m/z* 152 peak, the relative intensities of the *m/z* 94 and 96 peaks also distinguish the spectrum of cocaine from those of the other diastereomers. In the spectra of cocaine and pseudoallococaine, the peak at *m/z* 94 is more intense than the one at *m/z* 96; in the spectra of the other two isomers it is smaller than *m/z* 96 (compare Figures 4a and 14b). Spectra of D-labeled derivatives of cocaine and pseudococaine strongly indicate that this phenomenon is not just a simple reversal of intensities. The *m/z* 94 peak in the cocaine spectrum is significantly larger than that produced by pseudococaine, while the *m/z* 96 peaks in both spectra have similar intensities relative to those of other nearby peaks. In addition, the *m/z* 94 peak in the cocaine spectrum represents a greater proportion of the overall ion current (4.8%) than it does for the other isomers (2.9-3.3%) [5]. This implies that *m/z* 94 forms more easily with cocaine than it does with the other diastereomers.

None of the individual steps involved in generation of *m/z* 94b via either *m/z* 155 or 97 should be sensitive to stereochemical differences in the precursor ions because C2 and C3 are lost in forming this ion (Figures 2 and 23). However, a careful

examination of the other fragmentation pathways leading to *m/z* 94a show that formation of this ion is in competition with formation of *m/z* 152 via common intermediates: Pathways a + b vs. pathway c in Figures 9 and 10; and pathways a + c vs. pathway b in Figure 15. It is therefore tempting to conclude that the same factors that discourage formation of *m/z* 152 should encourage formation of *m/z* 94, and vice versa [5]. Indeed, the percent of total ion current for the *m/z* 94 peak in the spectra of the four diastereomers is virtually opposite of what is seen for the *m/z* 152 peak – that is, cocaine and pseudoallococaine produce the smallest *m/z* 152 peaks and the largest *m/z* 94 peaks, while for pseudococaine and allococaine the situation is reversed. One may conclude, then, that the relative rates of cyclic loss of benzoic acid for these compounds directly affect the ease of forming *m/z* 152 and thereby indirectly affect formation of *m/z* 94.

***m/z* 83.** The *m/z* 83 ion was identified as the most abundant fragment ion produced by the M^+ at low collision energies, indicating that it is formed directly in one step (Table II). The N-methyl group is retained, as are most of the H atoms in the five-membered ring portion of the molecule. Carbon atoms 2, 3, and 4 and their substituents are lost in forming this ion. This ion fragments almost exclusively to give *m/z* 82 (Figure 2) [1,2,13].

***m/z* 82.** Several important precursors to *m/z* 82 were identified by MS/MS, so that a number of pathways for its formation are likely. At low collision energies, the M^+ , *m/z* 182, *m/z* 97, and *m/z* 83 all can produce this ion, although *m/z* 303 and *m/z* 182 appear to be the most important. The pattern of D losses is similar to that seen with *m/z* 83, indicating that the structure of this ion consists of the five-membered ring of the tropane skeleton (Figure 2) [1,2,13]. A possible mechanism for formation directly from the M^+ is shown in Figure 24. Formation from *m/z* 182 is shown in Figure 21.

***m/z* 77.** This ion is a known fragment of *m/z* 105 (Figure 3). See discussion under *m/z* 122b above.

***m/z* 68.** The ion having *m/z* 96 is the only one that produces *m/z* 68 as a major fragment in the MS/MS experiments. The structure of *m/z* 68 appears to lack C2 and C3 and their substituents, which is consistent with the structure proposed for *m/z* 96. The only label that is clearly retained is the one on the N-methyl group, although it appears that several of the D atoms on C1, C5, C6, and C7 are retained as well (the observed cluster of peaks between *m/z* 68 and 73 in the spectrum of the d_6 -derivative shows no single important peak). Cyclic loss of ethylene from *m/z* 96 could account for formation of this ion, which most likely has a methylene-azacyclopropenium structure (Figure 23).

***m/z* 67.** The peaks at *m/z* 67 and *m/z* 68 often move together in the spectra of the D-labeled derivatives, which gives the impression that they are related to each other in a simple manner. Although the elemental composition for this ion was not determined, it appears as an important product ion in the MS/MS spectra of *m/z* 94 and *m/z* 82, indicating that it may have a different structure from *m/z* 68. It also appears that the

N-methyl label is lost, although in this area the spectrum of the N-CD₃ derivative shows more complexity than that simple analysis implies. On the other hand, formation of *m/z* 67 from *m/z* 82 can occur by loss of a methyl radical – presumably that on the N atom (Figure 24). The *m/z* 67 ion could form from *m/z* 94 by loss of HCN by a complex mechanism involving transfer of H atoms from the N-methyl group back onto the remaining C atoms (structure and mechanism not shown). This ion has a different elemental composition than that of the one formed from *m/z* 82. However, the presence of two separate mechanisms is not inconsistent with the D loss pattern.

***m/z* 59.** Only two of the studied ions give *m/z* 59 as a product ion – *m/z* 182 at higher, and *m/z* 152 at lower, collision energies. The only D label retained is on the O-CD₃ group. That, in combination with the elemental composition, indicates that this ion is the carbomethoxy ion. It is difficult to write a mechanism for its formation from either of these ions without invoking high-energy intermediates or significant rearrangement. Other precursors to this ion are possible.

***m/z* 55.** Of the listed precursors to the *m/z* 55 ion, only *m/z* 82 is important at low collision energies. This involves the loss of 27 u as C₂H₃. The spectra of D-labeled derivatives indicate loss of C2, C3, and C4. The pattern of loss shown by the *d*₆-derivative is complex and suffers from interference by the surrounding peaks, but at least some (and perhaps most) of the labels are retained. This is consistent with the fact that *m/z* 82 is the precursor. One possible mechanism is shown in Figure 24.

***m/z* 51.** This ion is a known fragment of *m/z* 105 and *m/z* 77 (Figure 3). See discussion under *m/z* 122b above.

***m/z* 42.** The only D labels retained by this ion are in the N-CD₃ group and one of the D atoms on C1, C5, C6, or C7. The *m/z* 96 ion is the only one studied that gives *m/z* 42 as a significant product ion at low collision energies. A possible mechanism is shown in Figure 23.

Conclusions

Although previously proposed mechanisms for formation of many of the more abundant ions in the cocaine spectrum were confirmed in this study, details about other fragmentations were revealed. Of particular interest are explanations clarifying the subtle differences between the spectra of the cocaine diastereomers – formation of *m/z* 152 from *m/z* 274 and 181 via pathways that are sensitive to the relative stereochemistry at C2 and C3, and the apparent inverse relationship between ease of formation of *m/z* 152 and *m/z* 94. Also of interest are a) the complexities that underlie the *m/z* 166 peak; b) the importance of the N-containing ion having *m/z* 122; and c) details regarding the formation of lower abundance ions, especially those above *m/z* 200.

Acknowledgements

The authors are deeply indebted to Donald A. Cooper, Senior Forensic Chemist, DEA Special Testing and Research Laboratory, ret., for obtaining the MS/MS and HR-MS data and for his ideas and comments on the manuscript.

References

1. Blossey EC, Budzikiewicz H, Obashi M, Fodor G, Djerassi C. Mass spectrometry in structural and stereochemical problems – XXXIX: Tropane alkaloids. *Tetrahedron* 1964;20:585-93.
2. Smith DH, Duffield AM, Djerassi C. Mass spectrometry in structural and stereochemical problems – CCXXII: Delineation of competing fragmentation pathways of complex molecules from a study of metastable ion transitions in deuterated derivatives. *Org Mass Spectrom* 1973;7:367-81.
3. Smith RM. The mass spectrum of cocaine. *J Forensic Sci* 1977;42(3):475-80.
4. Smith RM. Understanding mass spectra – A basic approach, 2nd ed., John Wiley and Sons, New York, NY 2004:262-74.
5. Cooper DA. Mass spectrometry in drug research. *Spectra* 1985;10(3):14-20.
6. Casale JF, Lewin AH, Raney HT, Cooper DA. Synthesis of deuterium labeled cocaine and pseudococaine. *J Labelled Compd Radiopharm* 1991;29(3):327-35.
7. Casale JF. A practical total synthesis of cocaine's enantiomers. *Forensic Sci Int* 1987;33(3):275-98.
8. McLafferty FW, Turecek F. Interpretation of Mass Spectra, 4th ed., University Science Books, Mill Valley, CA 1993:112-14.
9. Shapiro RH, Amenta DS, Kinter MT, Tomer KB. Mass spectral analysis of cocaine and pseudococaine. *Spectrosc Int J* 1983;2:227-31.
10. Rose ME. A caution on the use of metastable ions to assign fragmentation pathways. *Org Mass Spectrom* 1981; 16:323-24.
11. McLafferty FW, Turecek F. *op. cit.* 1993:115-20.
12. McLafferty FW, Turecek F. *op. cit.* 1993: 343-5.
13. Dewhurst JE, Kaminski JJ, Supple JH. Mass spectra of some tropane and tropidine derivatives. *J Heterocycl Chem* 1972;9(3):507-11.
14. Casale JF, Smith RM, unpublished results.
15. Jindal SP, Lutz T. Ion cluster techniques in drug metabolism: Use of a mixture of labeled and unlabeled cocaine to facilitate metabolite identification. *J Anal Toxicol* 1986;10 (4):150-5.
16. Ethier JC, Neville GA. Quadrupole EI and CI mass spectra of some principal tropane alkaloids. *Can J Spectrom* 1986;31:81-8.
17. Casale JF. Methyl esters of ecgonine: Injection-port produced artifacts from cocaine base (crack) exhibits. *J Forensic Sci* 1992;37(5):1295-310.
18. Vollmer D, Rempel DL, Gross ML, Williams F. Isomerization of 4-vinylcyclohexene radical cation: A tandem mass spectrometry study. *J Am Chem Soc* 1995;117:1669-70.
19. Zhang JY, Foltz RL. Cocaine metabolism in man: Identification of four previously unreported cocaine metabolites in human urine. *J Anal Toxicol* 1990;14(4):201-5.
20. Allen AC, Cooper DA, Kiser WO, Cottrell RC. The cocaine diastereomers. *J Forensic Sci* 1981;26(1):12-26.
21. Beynon JH, Saunders RA, Williams AE. *The Mass Spectra of Organic Molecules*, Elsevier, New York, NY, 1968:223.