"The Lost Manuscript" - A Posthumous Publication of:

The Discoloration of Illicit Drug Samples

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ABSTRACT: Discoloration (browning) of illicit cocaine exhibits during long-term storage is a common but not universal phenomenon. In order to gain a better understanding of the discoloration process(es), already discolored seized samples, and a wide variety of authentic drug mixtures that similarly discolored under long-term ambient and accelerated temperature - humidity studies, were subjected to acid base workup, column chromatography, and in-depth analyses of the pertinent fractions by EI and CI GC/MS, UV/Vis, and IR. The discolored samples were all found to contain a primary aromatic amine (either procaine or benzocaine), a sugar (either lactose or dextrose), and an acid (such as cocaine hydrochloride, boric acid, benzoic acid, etc.) The rate of discoloration of the drug mixtures was both pH and temperature dependant, i.e., the rate of sample browning increased with lower pH and/or higher temperature. All discolored samples that contained procaine or benzocaine also contained N-formylprocaine or N-formylbenzocaine, respectively, and these are therefore *bona fide* "marker" compounds for the browning of illicit cocaine. These derivatives are believed to be formed following the degradation of lactose or dextrose to 5-hydroxymethylfurfural, which in turn degraded to formic and levulinic acids; subsequent formylation of procaine or benzocaine gave the respective "marker" compounds. A number of highly colored compounds (yellow, blue, purple, and pink) were observed in column and thin-layer chromatography of the discolored samples, and are responsible for the sample discoloration. These compounds were not identified, but are believed to derive from condensation reactions between 5-hydroxymethylfurfural with the various amines in the samples.

KEYWORDS: Cocaine Hydrochloride, Discoloration, 5-Hydroxymethylfurfural, Procaine, Benzocaine, N-Formylprocaine, N-Formylbenzocaine, Lactose, Dextrose, Forensic Chemistry

[Foreword by the Corresponding Author and the *Microgram* Editor: This manuscript was authored in 1974 by then Senior Forensic Chemist Jim Moore; it was intended and formatted for publication in *Microgram*, but apparently was never submitted. It was re-discovered on July 14, 2008 by the co-author, Senior Research Chemist John Casale. It is published here verbatim (including 1970's formatting and scientific abbreviations), except that some experiments were repeated by the corresponding author to re-create legible figures, the above Abstract and Keyword set were provided by the Editor, and the layout was slightly reformatted by the Editor for improved readability.]

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Introduction

This paper reports the results of a two-year investigation studying the discoloration of illicit drug samples. The study was initiated as a result of several forensic laboratories reporting problems associated with the discoloration, or "browning," of illicit cocaine samples over a prolonged period of time. This "browning" phenomenon was of forensic interest in that there were discrepancies in the chemist's description of the sample prior to analysis and later, upon identifying the sample during court testimony. In most cases, the samples were white when first examined but subsequently acquired a brown coloration by the time the samples were reopened for court proceedings. This change in coloration caused significant problems for the chemists in that it could be assumed that a sample mix-up had occurred, and the disposition of the case would be in doubt based upon this reasoning. Dugar, *et al.* [1] have investigated this discoloration phenomenon in contraband cocaine.

The study in this paper satisfactorily characterizes the "browning" phenomenon occurring in certain illicit samples. This characterization is based upon: (A) a thorough literature review that describes the discoloration of legitimate pharmaceutical preparations, (B) in-depth analyses of illicit samples known to have undergone the discoloration process, (C) ambient and accelerated temperature-humidity studies conducted on authentic drug mixtures, and (D) the isolation and characterization of signature compounds associated with the discoloration process in illicit and authentic samples.

A. *Literature Review*

There has been considerable study of the discoloration associated with legitimate pharmaceutical products. Blaug and Huang [2,3] have described the discoloration of amphetamine sulfate - spray dried lactose and amphetamine sulfate - dextrose mixtures when subjected to elevated temperatures. In these studies, a product of sugar decomposition, namely 5-hydroxymethylfurfural (5-HMF), was suggested to be present. Dugar *et al.* [1] also reported the presence of 5-HMF in sugar-containing illicit samples that underwent discoloration. Castello and Mattocks [4] and Duvall *et al.* [5,6] reported interaction of various primary amines with lactose and dextrose and the subsequent discoloration of such samples. In these studies, the rate of "browning" was found to be dependant upon temperature and pH. Several investigators had studied the role of 5-HMF and related substances in the discoloration of amine - sugar mixtures [7-11]. Brownley and Lachman [12] studied the formation of 5-HMF in spray-dried lactose as well as conventionally-processed lactose.

The above-referenced studies clearly demonstrated that when mixtures of primary amines (amphetamine was most often studied) and certain sugars (lactose and dextrose) were subjected to elevated temperature and humidity conditions, significant discoloration occurred. Several authors reported a relationship between the rate of discoloration and pH of the sample. Finally, most of the studies reported a relationship between drug mixtures that had discolored and the presence of 5-HMF.

B. *In-Depth Analyses of Illicit Samples*

A number of illicit drug samples known to have undergone the discoloration process were obtained from various forensic laboratories. All samples were subjected to in-depth analyses for drug constituents as well as diluents. These analyses revealed that the samples had the following elements in common: (1) all samples contained cocaine hydrochloride, (2) all samples had undergone a white-to-brown color transition after prolonged storage under unspecified conditions, (3) all samples contained either lactose or dextrose, (4) all samples contained a primary aromatic amine, such as benzocaine, and (5) all samples were acidic in nature; this acidity was due, in part, to the presence of substances such as cocaine HCl, boric acid, etc. The results of the in-depth analyses indicated a positive correlation between sample composition and the discoloration process described by other investigators.

C. *Ambient and Accelerated Stability Studies of Authentic Samples*

A large number of authentic samples were prepared for stability studies (see Table I). The composition of these samples was based upon elements believed responsible, in part, for the discoloration process. The authentic samples were studied under ambient conditions for about two years and under accelerated conditions for about two months.

The primary amines used in this study were benzocaine and procaine HCl. These are two widely-used adulterants associated with illicit cocaine and heroin samples, respectively. The sugars used were lactose and dextrose. Preliminary studies indicated that mannitol and sucrose did not contribute significantly to the "browning" process. Since the literature review suggested a difference in the "browning" rates of spray-dried and conventionally-processed lactose, both were used in this study. The pH of the samples was controlled over an acid range by the introduction of substances of varying acidity. In order of increasing pKa, these substances were: oxalic acid, citric acid, benzoic acid, cocaine HCl, boric acid, heroin HCl, and procaine HCl. The concentration of all compounds was varied over a wide range.

In the ambient study, the authentic samples listed in Table I were placed in glass vials with screw-on plastic caps and stored in the dark. At the end of a two-year period, the samples were examined for discoloration. Table II lists those samples that exhibited significant discoloration. The samples listed in Table I, and not included in Table II, did not exhibit significant discoloration.

A review of Table II reveals that all samples that discolored contained either lactose or dextrose, benzocaine or procaine HCl, and were distinctly acidic. It should be noted that the concentration of the acidic component did not influence the discoloration significantly. On the other hand, the majority of the samples that did discolor contained the more acidic components, (i.e., oxalic, citric, and benzoic acids). Though the cocaine HCl - benzocaine - dextrose mixtures were of higher pH values, these samples also discolored. Though the explanation for this is not clear, it may be due to decomposition of cocaine with subsequent formation of benzoic acid resulting in a decrease of pH. It is also apparent from Table II that for a sample of given acidity, benzocaine - dextrose mixtures discolored more rapidly than benzocaine - lactose mixtures. Such a distinction is not apparent for procaine-containing samples.

The samples listed in Table I were also subjected to accelerated conditions for 61 days. The humidity varied from 50 - 70%, while the temperature was gradually increased from ambient to 48°C. Fig. 1 illustrates the results of the accelerated study. As the samples discolored significantly (brown to dark brown), they were removed from the temperature-humidity chamber and noted in Fig. 1. Due to the large number of samples that discolored, only a representative cross-section are noted in Fig. 1.

The results of the accelerated stability studies can be summarized as were the ambient study described previously. Additionally, there does exist a positive correlation between the rate of discoloration of the authentic samples and increases in temperature. There is no apparent difference in the discoloration rates of samples containing the various grades of lactose, including spray-dried and conventionally-processed.

Some of the samples subjected to the accelerated study showed no significant discoloration. These included most of the two-component samples as well as those three-component mixtures that did not contain either a sugar, a primary amine, or a strong acid. Those samples that apparently contained the necessary components for "browning," but showed no significant discoloration, were generally of a higher pH, and usually contained lactose as the sugar diluent. These included samples containing lactose mixed with heroin HCl - procaine HCl, heroin HCl - benzocaine, boric acid - procaine HCl, boric acid - benzocaine, cocaine HCl - procaine HCl, and cocaine HCl - benzocaine. In general, samples containing dextrose were found to discolor at a significantly faster rate than those containing lactose.

Table I - Composition of Authentic Samples Subjected to Ambient and Accelerated Studies [a]

1.   2% Ox - 49% Bnzn - 49% Lac
2. 25% Ox - 37% Bnzn - 37% Lac
3. 70% Ox - 15% Bnzn - 15% Lac
4.   2% Ox - 49% Proc - 49% Dex
5. 25% Ox - 37% Proc - 37% Dex
6. 70% Ox - 15% Proc - 15% Dex
7.   2% Ox - 49% Bnzn - 49% Dex
8. 25% Ox - 37% Bnzn - 37% Dex
9. 70% Ox - 15% Bnzn - 15% Dex
10. 25% Ox - 37% Proc - 37% Lac
11. 2% Ox - 49% Proc - 49% Lac
12. 70% Ox - 15% Proc - 15% Lac
13. 2% Cit - 49% Proc - 49% Dex
14. 25% Cit - 37% Proc - 37% Dex
15. 70% Cit - 15% Proc - 15% Dex
16. 2% Cit - 49% Bnzn - 49% Dex
17. 25% Cit - 37% Bnzn - 37% Dex
18. 70% Cit - 15% Bnzn - 15% Dex
19. 2% Cit - 49% Proc - 49% Lac
20. 25% Cit - 37% Proc - 37% Lac
21. 70% Cit - 15% Proc - 15% Lac
22. 2% Cit - 49% Bnzn - 49% Lac
23. 25% Cit - 37% Bnzn - 37% Lac
24. 70% Cit - 15% Bnzn - 15% Lac
25. 2% Bzoc - 49% Proc - 49% Dex
26. 25% Bzoc - 37% Proc - 37% Dex
27. 70% Bzoc - 15% Proc - 15% Dex
28. 2% Bzoc - 49% Bnzn - 49% Dex
29. 25% Bzoc - 37% Bnzn - 37% Dex
30. 70% Bzoc - 15% Bnzn - 15% Dex
31. 2% Bzoc - 49% Proc - 49% Lac
32. 25% Bzoc - 37% Proc - 37% Lac
33. 70% Bzoc - 15% Proc - 15% Lac
34. 2% Bzoc - 49% Bnzn - 49% Lac
35. 25% Bzoc - 37% Bnzn - 37% Lac
36. 70% Bzoc - 15% Bnzn - 15% Lac
37. 2% Coc - 49% Proc - 49% Dex
38. 25% Coc - 37% Proc - 37% Dex
39. 70% Coc - 15% Proc - 15% Dex
40. 2% Coc - 49% Bnzn - 49% Dex

41. 25% Coc - 37% Bnzn - 37% Dex
42. 70% Coc - 15% Bnzn - 15% Dex
43. 2% Coc - 49% Proc - 49% Lac
44. 25% Coc - 37% Proc - 37% Lac
45. 70% Coc - 15% Proc - 15% Lac
46. 2% Coc - 49% Bnzn - 49% Lac
47. 25% Coc - 37% Bnzn - 37% Lac
48. 70% Coc - 15% Bnzn - 15% Lac
49. 2% Bor - 49% Proc - 49% Dex
50. 25% Bor - 37% Proc - 37% Dex
51. 70% Bor - 15% Proc - 15% Dex
52. 2% Bor - 49% Bnzn - 49% Dex
53. 25% Bor - 37% Bnzn - 37% Dex
54. 70% Bor - 15% Bnzn - 15% Dex
55. 2% Bor - 49% Proc - 49% Lac
56. 25% Bor - 37% Proc - 37% Lac
57. 70% Bor - 15% Proc - 15% Lac
58. 2% Bor - 49% Bnzn - 49% Lac
59. 25% Bor - 37% Bnzn - 37% Lac
60. 70% Bor - 15% Bnzn - 15% Lac
61. 2% Her - 49% Proc - 49% Lac
62. 25% Her - 37% Proc - 37% Lac
63. 70% Her - 15% Proc - 15% Lac
64. 2% Her - 49% Bnzn - 49% Lac
65. 25% Her - 37% Bnzn - 37% Lac
66. 70% Her - 15% Bnzn - 15% Lac
67. 2% Her - 49% Proc - 49% Dex
68. 25% Her - 37% Proc - 37% Dex
69. 70% Her - 15% Proc - 15% Dex
70. 2% Her - 49% Bnzn - 49% Dex
71. 25% Her - 37% Bnzn - 37% Dex
72. 70% Her - 15% Bnzn - 15% Dex
73. 25% Coc - 25% Bnzn - 50% Ox
74. 25% Coc - 25% Proc - 50% Ox
75. 25% Her - 25% Proc - 50% Ox
76. 50% Proc - 50% Ox
77. 50% Bnzn - 50% Ox
78. 50% Dex - 50% Ox
79. 50% Lac - 50% Ox
80. 50% Coc - 50% Ox
81. 50% Her - 50% Ox

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[a] Key to Abbreviations: Ox = oxalic acid, Cit = citric acid, Bzoc = benzoic acid, Coc = cocaine HCl, Bor = boric acid, Her = heroin HCl, Proc = procaine HCl, Bnzn = benzocaine, Dex = dextrose, Lac = lactose. Various grades of lactose, including spray-dried and conventionally processed were used.

Table II - Authentic Samples that Exhibited Discoloration
after a Two-Year Study under Ambient Conditions [a]

<u>Sample Number</u> [b]	<u>Sample Appearance</u>
4, 5, 6, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 20, 21, 27, 28, 29, 30, 41, 42	Uniform dark brown to black
1, 2, 3, 7, 8, 9, 23, 24, 25, 26, 32, 33, 40 49, 50, 52, 53	Uniform light to medium brown
16, 22, 23	Dark specks in white powder

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[a] Ambient conditions: avg. room temperature = 24 - 26°C; humidity range = 60 - 80% RH.
[b] Refer to Table I for sample composition.

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Discoloration of Authentic Samples during Accelerated Study
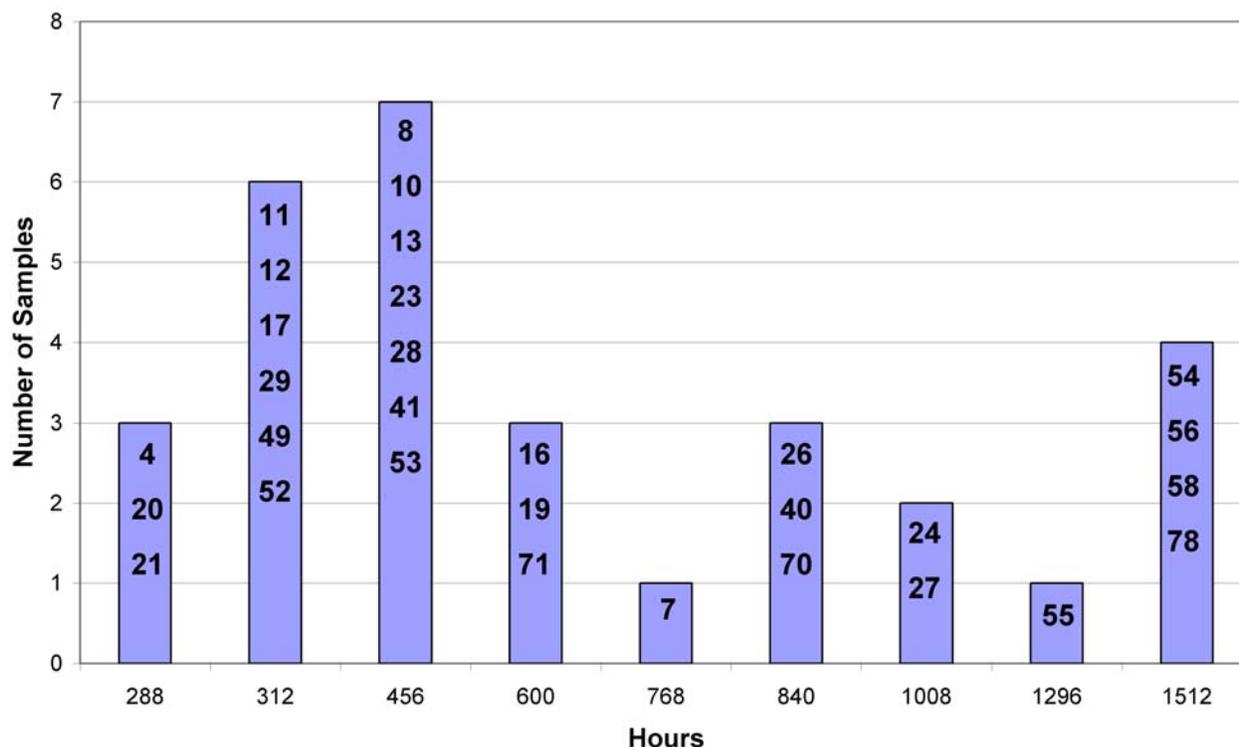
Figure 1: Graph illustrating discoloration of authentic samples subjected to accelerated conditions.
As samples discolored, they were removed from temperature-humidity chamber and noted on graph.
Refer to Table I for sample composition.

In summary, the results of the ambient and accelerated studies have established a clear relationship between the discoloration of illicit samples and their composition and storage conditions. Despite this positive correlation, full characterization of this "browning" phenomena would not be complete unless signature compounds that resulted as by-products of the discoloration process were isolated and identified. This work is described below.

D. *Isolation and Characterization of Signature Compounds*

1. Chemicals and Solvents

   The formic acid used in this study was 88% analytical reagent grade and obtained from Mallinkrodt Chemical Works (St. Louis, Missouri). The deuterated formic acid (DCOOH) was 99% atom % D and was supplied by Merck and Co., Inc. (St. Louis, Missouri). All other chemicals and solvents used were of high quality and obtained from the usual commercial sources.

2. Drug Standards

   All drug materials were provided by the Special Testing and Research Laboratory, Drug Enforcement Administration.

3. Chromatographic Materials

   (a) The chromatographic partitioning columns were 250 mm in length x 22 mm i.d., and obtained from Kontes Glass Co. (Vineland, New Jersey).

   (b) The diatomaceous earth used in the partitioning work was Celite 545 acid-washed (AW), and obtained from Johns-Manville Co. (Inglewood Cliff, New Jersey).

   (c) All thin layer chromatography was done on glass plates coated with silica gel GF (250 or 2000μ thickness). These plates were obtained from Analtech, Inc. (Newark, Delaware).

   (d) All gas chromatographic columns were obtained from Applied Science Laboratories (State College, Pennsylvania) (see body of paper for dimensions). The various column packings were also obtained from Applied Science Laboratories. These included 3% OV-1 and 3% OV-25, all on Chromosorb WHP (100-120M). All internal standards were also obtained from Applied Science Laboratories.

4. Instrumentation

   (a) Gas Chromatography - The gas chromatograph work was done on a Packard 7400 gas chromatograph equipped with a flame ionization detector (FID) (see body of paper for other GLC parameters).

   (b) Ultraviolet Spectrometry (UV) - All UV spectra were recorded on a Cary 14 spectrophotometer.

   (c) Infrared Spectrometry (IR) - All IR spectra were recorded on a Perkin-Elmer 457 spectrophotometer.

   (d) Gas Chromatography - Mass Spectrometry (GC-MS) - A Finnigan 4000 mass spectrometer was used in this study. It was interfaced with a Finnigan 9610 GC and Finnigan 6110 data system. The gas chromatograph was equipped with a 6 ft. x 2 mm i.d. glass column packed with 3% OV-1 and Gas Chrom Q (80-100M).

   All electron impact (EI) spectra were acquired under the following conditions: emission current - 0.35 mA, amplifier sensitivity - 10 - 8 A/V, electron energy - 70 eV, and electron multiplier - 1600 V. The carrier gas was Helium and maintained at a flow rate of about 20 cc/min. The column temperature was programmed between 150 and 250°C, while the ionizer, separator, and transfer line temperatures were maintained at 250, 260, and 260°C, respectively.

All chemical ionization (CI) spectra were obtained under the following conditions: Helium was the carrier gas and methane was used as the reactant gas; the ionizer was maintained at a pressure of about 0.40 torr and a temperature of 200°C; all other parameters are the same as for the EI study.

5. Isolation of Signature Compounds

During the in-depth analyses of the discolored, illicit samples described previously, trace amounts of unidentified impurities were detected. The methodology described below was developed in order to isolate these impurities in sufficiently pure form for spectroscopic characterization.

(a) *Isolation of Signature Compounds in Illicit and Authentic, Brown Samples Containing Benzocaine*

About 0.5 cc of 0.1N H_2SO_4 is mixed with 1 gm Celite 545 AW and packed moderately in a chromatographic partitioning column. An appropriate quantity of sample is dissolved in 2 cc of 0.1N H_2SO_4 and 3 gm of Celite 545 AW are added; after mixing, the sample is packed moderately above the bottom layer of the column. The column is eluted with about 50 - 75 cc of water-saturated ethyl ether. This eluate contains benzocaine and the signature compound. Cocaine and other basic drugs are retained by the column. The ether eluate is evaporated gently to dryness. Depending upon sample composition, this fraction may be sufficiently pure for spectroscopic characterization.

If additional "cleanup" is necessary, the following chromatographic procedure may be used. The residue obtained above from the ether eluate is triturated with 2 cc of 0.1N $NaHCO_3$; 3 gm of Celite 545 are added and mixed until fluffy; this mixture is packed moderately in a chromatographic partitioning column containing a layer of 0.5 cc 0.1N $NaHCO_3$ mixed with 1 gm of Celite 545 AW. The column is then eluted with 75 - 100 cc of water-saturated petroleum ether. This fraction consists primarily of benzocaine. The column is then eluted with 50 - 75 cc water-saturated ethyl ether. This fraction contains the signature compound and trace amounts of benzocaine. The ether eluate is evaporated carefully to dryness. The residue may be characterized spectroscopically or subjected to further purification using the TLC technique described below.

The residue obtained above is dissolved in a small volume of methylene chloride and spotted or streaked on a silica gel GF plate (250 or 2000µ thickness). The plate is developed with a solvent mixture of ethyl ether : petroleum ether (65:35). After development, the plate is dried and viewed under short wave UV. Both benzocaine and the signature compound appear as dark spots or bands at R_f values of about 0.45 and 0.17, respectively. The spot or band representing the signature compound is removed from the plate and placed in a small vial. Methanol is added to the vial and warmed gently on a steam bath. The vial is centrifuged and the methanol is decanted and evaporated carefully to dryness. The residue may be subjected to spectroscopic characterization.

Isolation of the signature compound may also be accomplished using GLC fraction collection techniques. Table III gives the appropriate GLC parameters and retention data for benzocaine, the signature compound, and internal standards.

The signature compound isolated by one or more of the chromatographic procedures given above is subjected to MS, UV, and IR characterization described later in this paper.

(b) *Isolation of Signature Compounds in Illicit and Authentic Brown Samples Containing Procaine*

An appropriate quantity of sample is dissolved in 2 cc of water; the solution is made basic with $NaHCO_3$ and 3 gm of Celite 545 AW are added; after uniform mixing, the sample is packed in a column containing a layer of 0.5 cc of 0.1N $NaHCO_3$ mixed with 1 gm of Celite 545 AW. The signature compound is

isolated following petroleum and ethyl ether elutions, as described above for samples containing benzocaine (ethyl ether eluate contains the signature compound as well as small quantities of procaine).

If necessary, additional TLC purification may be required as described above for benzocaine-containing samples. The adsorbent used is silica gel GF and the solvent system is ammonia-saturated chloroform : methanol (18:1). The R_f values for the procaine and the signature compound are about 0.8 and 0.6, respectively. (Note: using this solvent system, acetylprocaine and the signature compound have similar R_f values.)

Isolation of the signature compound may also be accomplished using GLC fraction collection techniques. Table IV gives the GLC parameters and retention data for procaine, the signature compound, and internal standard.

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Table III - GLC Data for Benzocaine, Internal Standards, and Signature Compound

	Retention Time (Min.)	
	3% OV-1 [a]	3% OV-25 [b]
Compound		
Benzocaine	2.2	1.8
Signature Compound	5.0	5.1
Eicosane	9.9	-
Hexacosane	-	9.2

[a] 6 ft. x ¼ in. i.d. column packed with 3% OV-1 on Chromosorb W HP (100 - 120M), injector temp. = 275°C, column temp. = 190°C, manifold temp. = 250°C, detector temp. = 250°C; N_2 carrier flow = 60 cc/min.

[b] 6 ft. x ¼ in. i.d. column packed with 3% OV-25 on Chromosorb W HP (100 - 120M), injector temp. = 275°C, column temp. = 205°C, manifold temp. = 250°C, detector temp. = 250°C; N_2 carrier flow = 60 cc/min.

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Table IV - GLC Data for Procaine, Internal Standard, and Signature Compound

	Retention Time (Min.)	
	3% OV-1 [a]	3% OV-25 [b]
Compound		
Procaine	3.8	2.6
Octacosane	-	4.3
Signature Compound	8.0	6.1

[a] 6 ft. x ¼ in. i.d. column packed with 3% OV-1 on Chromosorb W HP (100 - 120M), injector temp. = 275°C, column temp. = 220°C, manifold temp. = 250°C, detector temp. = 250°C; N_2 carrier flow = 60 cc/min.

[b] 6 ft. x ¼ in. i.d. column packed with 3% OV-25 on Chromosorb W HP (100 - 120M), injector temp. = 275°C, column temp. = 240°C, manifold temp. = 265°C, detector temp. = 265°C; N_2 carrier flow = 60 cc/min.

The signature compound isolated from procaine-containing samples by one or more of the chromatographic procedures given above are subjected to MS, UV, and IR identification outlined below.

E. *Identification*

1. <u>Signature Compound in Benzocaine-Containing Samples</u>

   (a) *Mass Spectral Analysis*

   The purified residue obtained above from benzocaine-containing samples was introduced into the GC-MS under conditions described earlier.

   The EI spectrum of the signature compound was rather simple and quite similar to the EI spectrum of benzocaine (Fig. 2a and 2b). Benzocaine and the signature compound yielded molecular ions at *m/e* 165 and *m/e* 193, respectively. Both compounds exhibited prominent ions at $(M-28)^+$, $(M-45)^+$, and $(M-73)^+$. In both compounds, an ion of moderate intensity was noted at *m/e* 65.

   The CI spectra of the signature compound confirmed the molecular weight of 193 obtained from the EI spectrum. This confirmation was supported by the presence in CI of an intense quasi-molecular ion at *m/e* 194 as well as the expected adduct ions at $(M+29)^+$ and $(M+41)^+$.

   The EI and CI data obtained above suggested the signature compound to be closely related to benzocaine, but substituted with a functional group of 29 mass units, such as an ethyl or formyl substituent.

   (b) *UV Analysis*

   In methanol, benzocaine yielded a UV maximum at 292 nm, while the signature compound gave a maximum at 269 nm. This hypsochromic shift of 23 nm would suggest that an electron withdrawing group had been introduced in the phenyl ring of benzocaine, probably *para-* to the carboxyethyl group. Inspection of the benzocaine molecule revealed that substitution on the highly reactive amino function would be a likely occurrence. The substitution of an ethyl group on the amino function would have an inductive effect which would probably increase the wavelength of UV maximum. However, the introduction of a formyl group would decrease the interaction of the lone pair of electrons of nitrogen with the phenyl ring resulting in a decrease in wavelength of UV maximum.

   Since MS and UV analyses suggested the signature compound to be N-formyl substituted benzocaine, an infrared analysis was done to support this postulation.

   (c) *Infrared Analysis* [12,13]

   The infrared spectra (KBr medium) of benzocaine and the signature compound were studied and found to be similar, but not identical. The IR spectrum of the signature compound supported a formyl substituent on the amino function of benzocaine. While benzocaine exhibited three bands between 3500 and 3200 cm^{-1} due to asymmetric, symmetric, and bonded NH$_2$ stretching vibrations, the signature compound exhibited only one intense band at 3310 cm^{-1}. This was strong evidence that substitution on the nitrogen function had occurred. This was further supported by the absence of the intense NH bending vibrational band found at 1630 cm^{-1} in primary aromatic amines such as benzocaine. The substituent on the nitrogen function was probably carbonyl as supported by an additional carbonyl band at 1700 cm^{-1} and a band at 1530 cm^{-1} due probably to the NH bending vibration found in amides (Amide II band).

The other bands in the spectrum of the signature compound were similar to benzocaine. These intense bands were at 1685 cm^{-1} and 1170 cm^{-1}, due to C-O stretching modes. Intense bands at 1595 cm^{-1} and 1505 cm^{-1}, were due to the phenyl moiety; and absorption bands between 850 cm^{-1} and 750 cm^{-1}, were due to C-H out-of-plane bending in the phenyl ring.

The combined UV, IR, and MS data suggested the signature compound to be N-formylbenzocaine (ethyl-*p*-formamidobenzoate). This structure is confirmed later in this paper by synthesis of the compound and its deuterated analogue and comparing its spectral data with that of the isolated signature compound. Additionally, a mechanistic interpretation of the mass spectral fragmentation of N-formylbenzocaine is presented.

2. Signature Compound in Procaine-Containing Samples

   (a) *Mass Spectral Analysis*

   The purified residue obtained above from samples containing procaine was introduced into the GC-MS under conditions described earlier.

   The EI spectra of the signature compound and procaine were similar (Fig. 3a and 3b). Both spectra yielded ions at *m/e* 58, 65, 71, 86, 92, 99, 120, and 137. Additionally, procaine yielded an ion at *m/e* 164, while the signature compound produced ions at *m/e* 148, 192, and 249. Since procaine did not yield a molecular ion, it was not surprising that the molecular ion was not detected for the signature compound.

   The CI spectrum of procaine yielded an intense quasimolecular ion at *m/e* 237 as well as the expected adduct ions at $(M+29)^+$ and $(M+41)^+$. The CI spectrum of the signature compound produced an intense quasimolecular ion at *m/e* 265 as well as the expected adduct ions at $(M+29)^+$ and $(M+41)^+$. These data suggested the molecular weight of the signature compound to be 264 amu.

   The EI and CI data suggested the signature compound to be related to procaine, but with an additional substituent of 29 mass units. Since N-formylbenzocaine had been identified above, the substituent in procaine was hypothesized to be a formyl group.

   b. *Ultraviolet Analysis*

   The UV maxima of procaine and the signature compound in methanol were at 295 and 271 nm, respectively. This supported an assignment of a formyl group as a substituent on the aromatic nitrogen function in procaine (for discussion, refer above to UV analysis of benzocaine-related signature compound).

   c. *Infrared Analysis*

   The infrared spectra of procaine base (KBr medium) and the signature compound base (salt plates) were studied (signature compound base is an oil). As in benzocaine, procaine base exhibits three intense bands between 3500 and 3200 cm^{-1} due to NH$_2$ stretching vibrational modes. In the signature compound, only one significant band appeared at 3300 cm^{-1}. However, unlike N-formylbenzocaine, this band was rather broad and of low to moderate intensity. This was probably due to the fact that hydrogen bonding occurred because the spectrum was obtained as an oil. Nonetheless, it was apparent that substitution on the aromatic amine function in procaine had occurred. This assignment was supported by the absence of
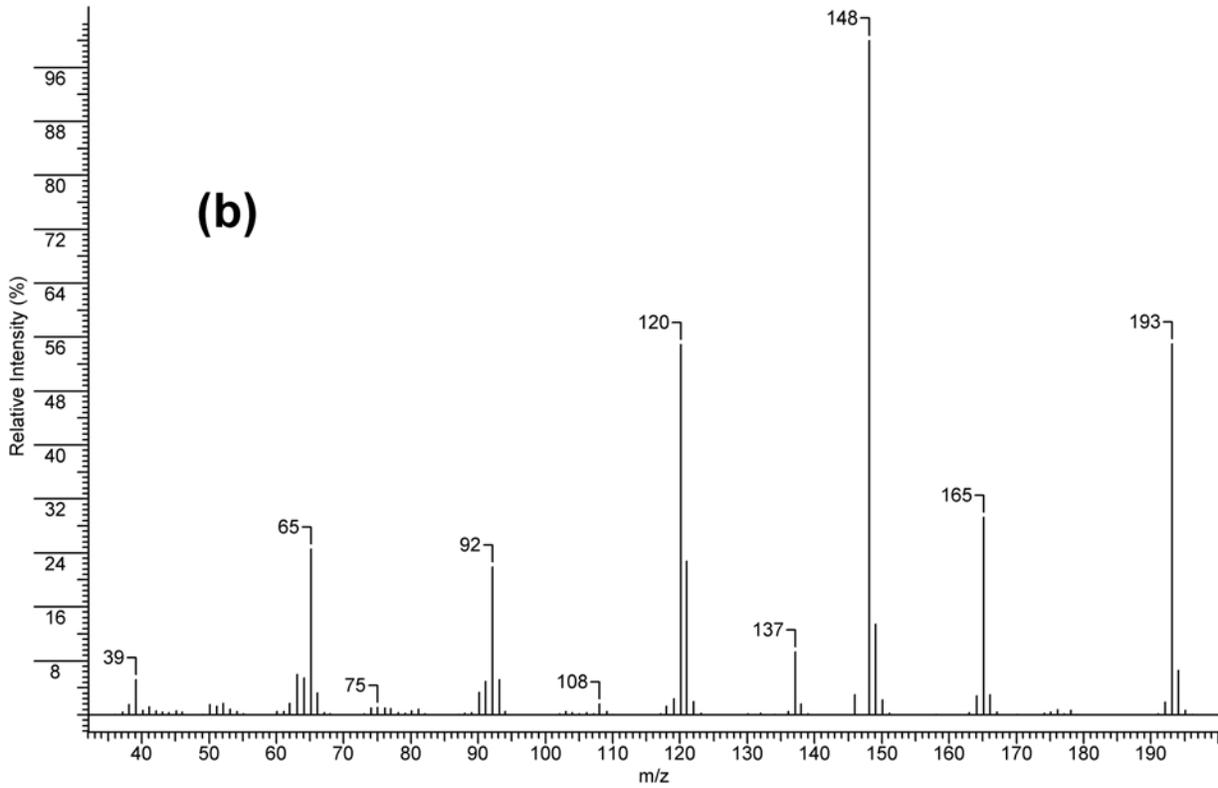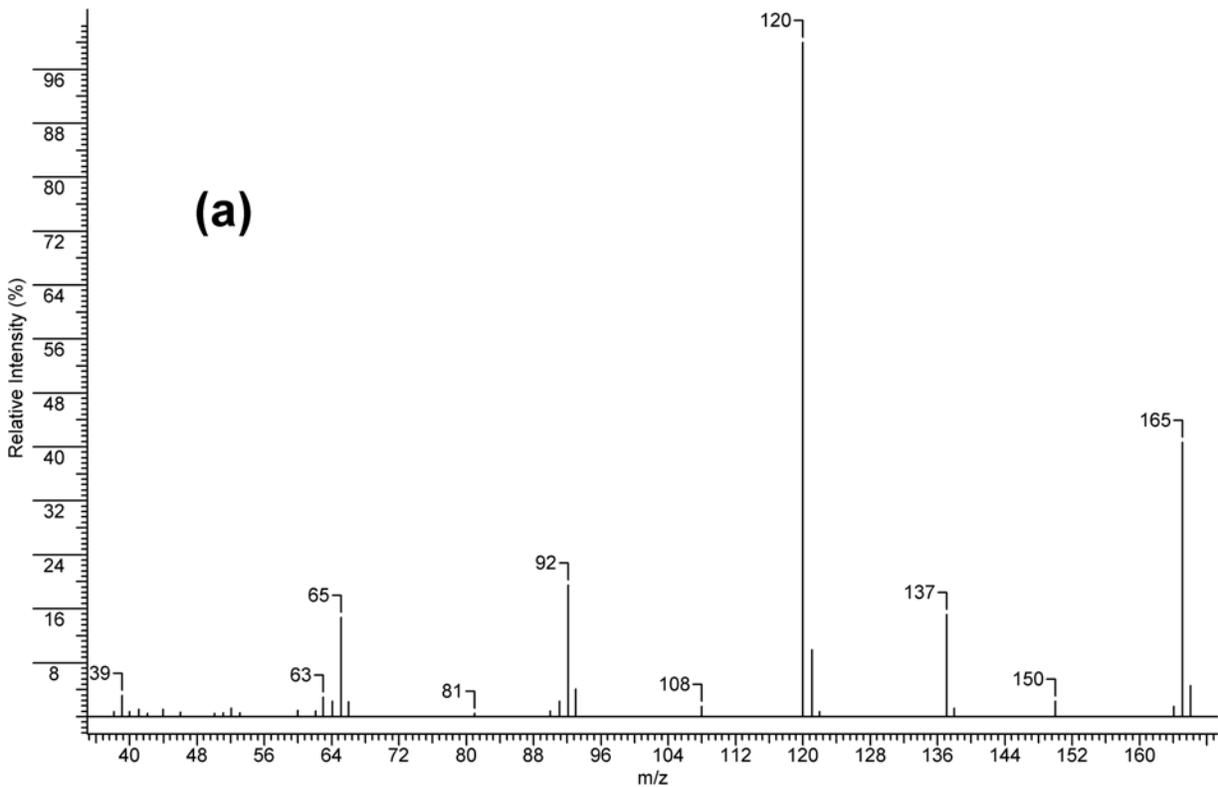
Figure 2: Spectra regenerated on GC-MSD. Conditions utilized were identical to those published in Microgram Journal 2006;4(1-4):47-53. (a) EI mass spectrum of benzocaine. (b) EI mass spectrum of N-formylbenzocaine.
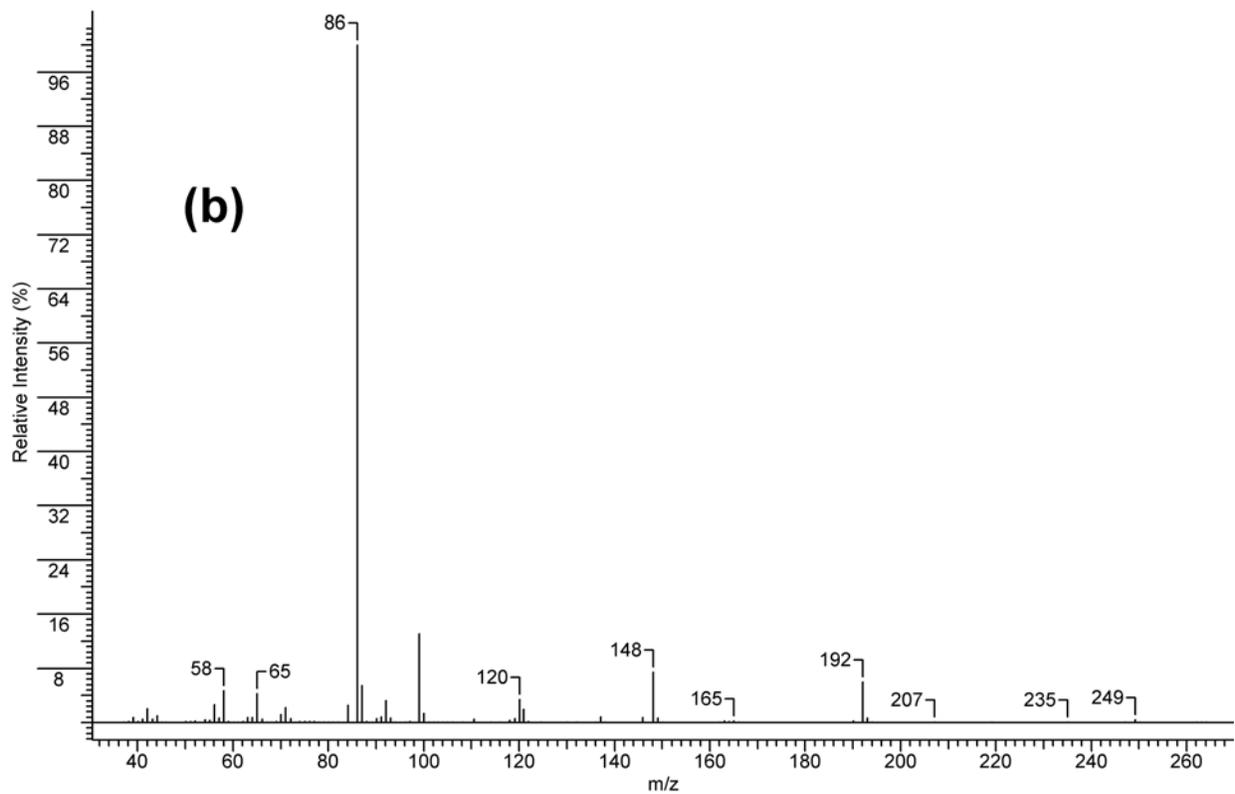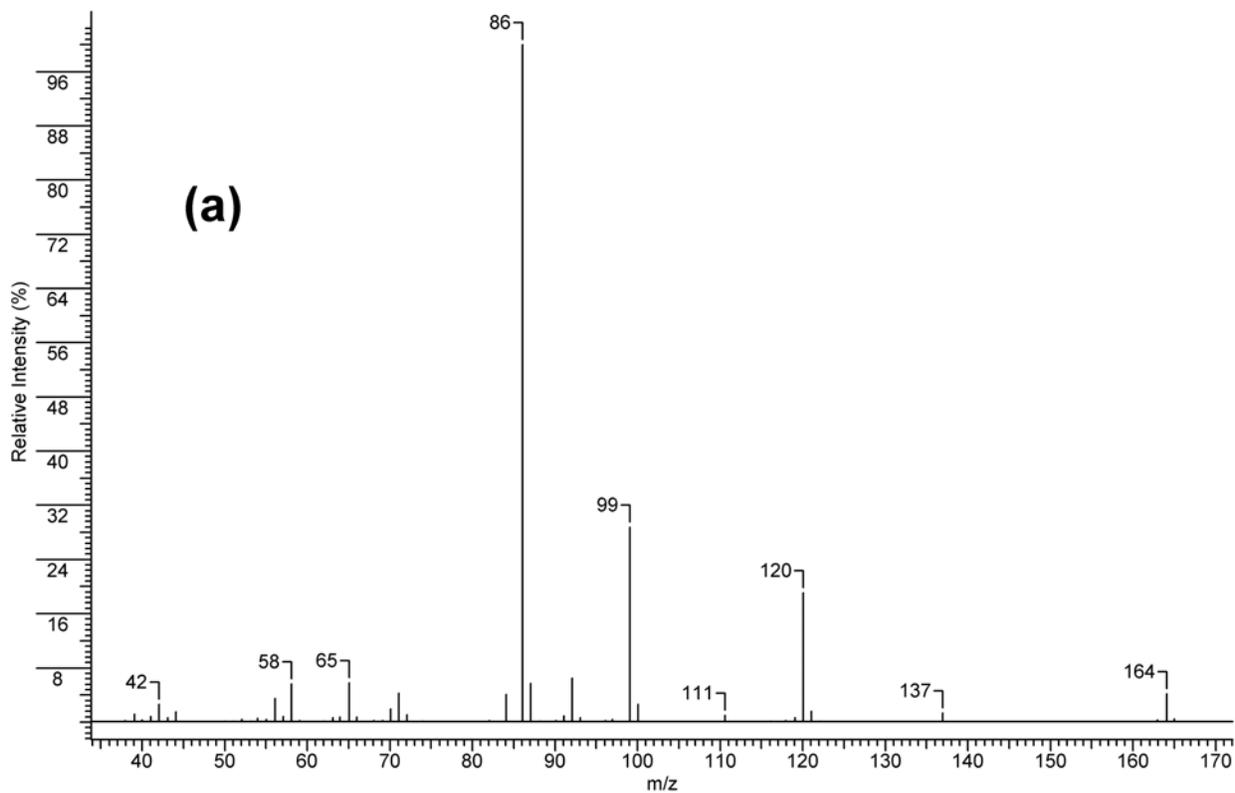
Figure 3: Spectra regenerated on GC-MSD. Conditions utilized were identical to those published in Microgram Journal 2006;4(1-4):47-53. (a) EI mass spectrum of procaine. (b) EI mass spectrum of N-formylprocaine.

the NH bending mode at 1620 cm^{-1} due to the NH$_2$ group in procaine. The N substituent is probably a carbonyl function, as supported by a very broad and intense band at 1700 cm^{-1}. This band represented a combination of two carbonyl bands, one due to the amido function and the other due to the ester moiety (note: acetylprocaine also exhibits one intense and broad band at about 1700 cm^{-1}). Further support for a carbonyl substituent on the aromatic nitrogen function arises from an intense band at 1530 cm^{-1} due to the NH bending mode found in amides (Amide II band). The other bands in the spectrum of the signature compound were similar to procaine base. These included bands at 1700 cm^{-1} due, in part, to the ester carbonyl stretching mode at 1270 and 1170 cm^{-1} due to the C-O stretching mode, an intense band at 1595 cm^{-1} due to the phenyl moiety, and absorption bands between 850 and 750 cm^{-1} due to C-H out-of-plane bending in the phenyl ring.

The combined UV, IR, and MS data suggested the signature compound, isolated from discolored authentic samples containing procaine, to be N-formylprocaine (2-diethylamino-*p*-formamidobenzoate). This structure was confirmed by the synthesis of this compound and its deuterated analogue and comparing its spectral data with that of the isolated signature compound. Additionally, a mechanistic interpretation of the mass spectral fragmentation of N-formylprocaine is given.

3. Synthesis of N-Formylbenzocaine, N-Formylprocaine, and their Deuterated Analogues

One gram quantities of benzocaine and procaine were dissolved in separate 10 cc portions of formic acid. One gram quantities of both compounds were also dissolved in separate 10 cc portions of deuterated formic acid (DCOOH). After about 20 hours at room temperature, the solutions were diluted with a large volume of ice water and made basic with NaHCO$_3$. The signature compounds and small amounts of unreacted benzocaine and procaine were extracted from the NaHCO$_3$ solution into ethyl ether. The ether extracts were passed through anhydrous sodium sulfate and evaporated to dryness. N-formylbenzocaine is a white solid, and N-formylprocaine is an oil. Both signature compounds were synthesized in greater than 90% yield.

The UV, IR, and MS spectral data for the synthesized standards were virtually identical with the signature compounds isolated from samples, thus confirming their identity as N-formylbenzocaine and N-formylprocaine.

4. Mechanistic Interpretation of Mass Spectral Fragmentation of N-Formylbenzocaine and N-Formylprocaine [14,15]

The N-formyl and deutero-formyl derivatives of benzocaine and procaine were synthesized as described above.

(a) *N-Formylbenzocaine* (Fig. 2b and 4)

The EI mass spectrum of N-formylbenzocaine yields a moderately intense molecular ion at *m/e* 193 (I). As expected, the deuterated analogue produced a molecular ion at *m/e* 194. An ion of moderate intensity at *m/e* 165 is due to expulsion of ethylene from the molecular ion with hydrogen transfer to the fragment ion (II). Though this ion could be rationalized as elimination of the formyl group with hydrogen transfer, the appearance of *m/e* 137 in benzocaine supports the former postulation. This assignment is confirmed by the appearance of an ion at *m/e* 166 in the deuterated species. The base peak at *m/e* 148 is due to elimination of C$_2$H$_5$O from the molecular ion with the fragment ion charge on the oxygen (III). A weak ion formed at *m/e* 137 is due to loss of CO and C$_2$H$_4$ with a double hydrogen transfer (IV). As expected, a shift of 1 amu occurred upon deuteration. A moderately intense ion at *m/e* 65 is probably due to the

cyclopentadienyl ion, an ion seen frequently in the spectra of aromatic amines (V). An intense ion found at m/e 120 (VI) is due to losses of C_2H_5O and the formyl group with the formyl hydrogen transferred to the charged species. Confirmation is obtained by the appearance of an intense ion at m/e 121 in the deuterated species. A moderately intense ion at m/e 92 is probably due to the amino phenyl moiety and involves hydrogen transfer from the expelled formyl group (VII). An ion at m/e 93 in the deuterated species confirms this postulation.

I. m/e 193

II. m/e 165

III. m/e 148

IV. m/e 137

V. m/e 65

VI. m/e 120

VII. m/e 92
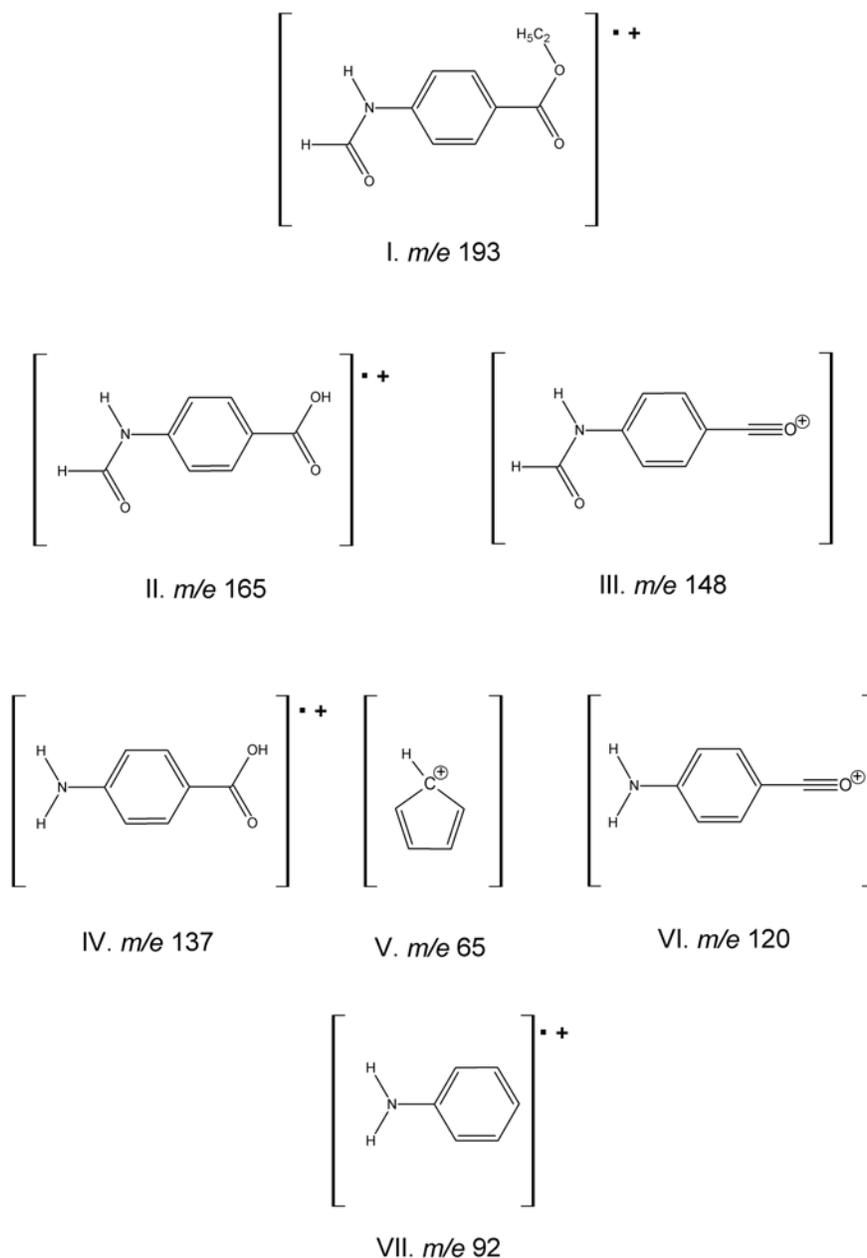
Figure 4: Prominent ions in EI mass spectrum of N-formylbenzocaine.

* * * * *

(b) *N-Formylprocaine* (Fig. 3b and 5)

N-Formylprocaine does not yield a detectable molecular ion under EI conditions. However, under CI conditions, an intense quasimolecular ion at *m/e* 265 is present (VIII). Under EI, a weak ion is observed at *m/e* 249 which shifts 1 amu upon deuteration (IX). This ion is due to the elimination of a methyl group from the diethylamino function in the parent molecule. An ion of low intensity occurs at *m/e* 192 and is due to elimination of the diethylamino function from the molecular ion (X). Upon deuteration, an expected shift of 1 amu is observed. An ion of moderate intensity is found at *m/e* 99 and does not shift upon deuteration. It is due to fission of C(1)-O bond with loss of hydrogen from C1 (XI). The most intense ion in the spectrum occurs at *m/e* 86 and is due to fission of the C(1)-C(2) bond with charge retention on the diethylamino moiety (XII). As expected, no shift was observed upon deuteration. Ions at *m/e* 120 and 148 can be rationalized as for N-formylbenzocaine (Fig. 4).

VIII. *m/e* 265 CI

IX. *m/e* 249 EI

X. *m/e* 192 EI

XI. *m/e* 99 EI

XII. *m/e* 86 EI
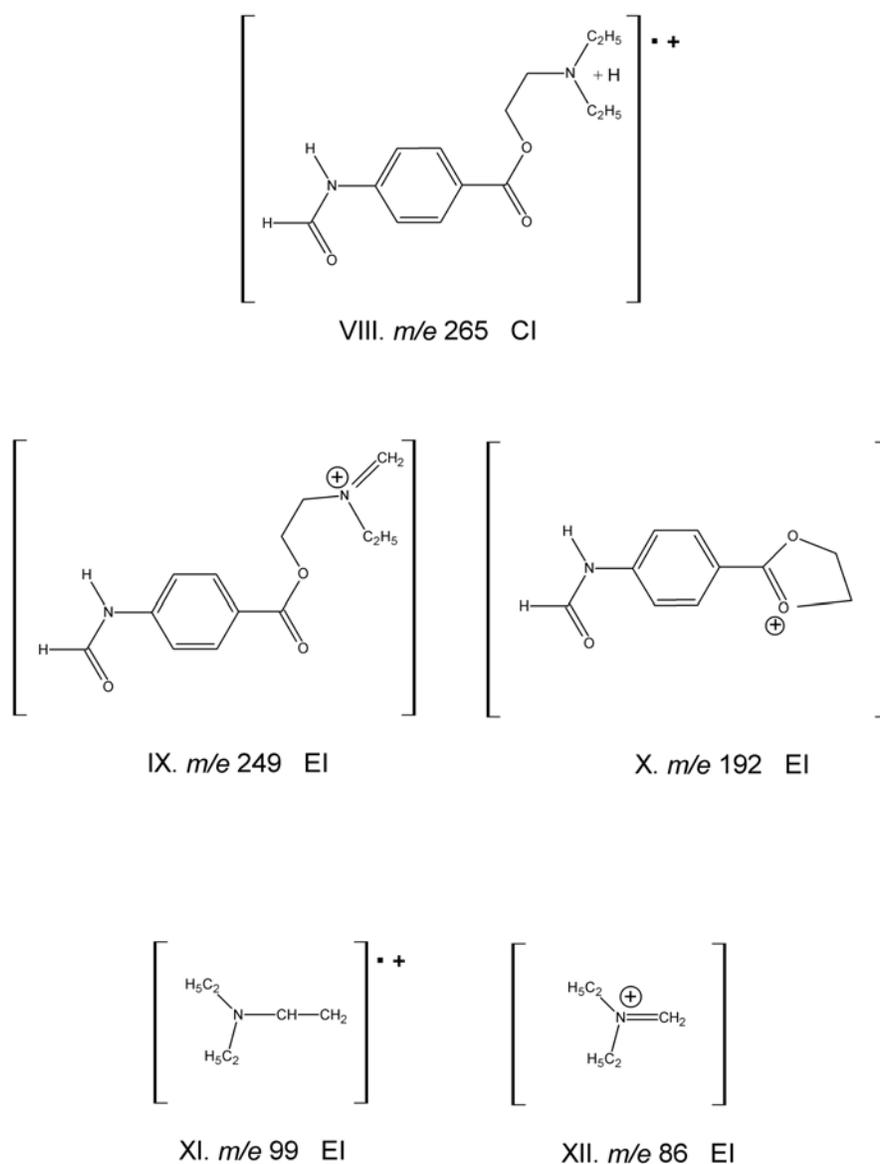
Figure 5: Prominent ions in the EI and CI mass spectrum of N-formylprocaine.

5. Evaluation of N-Formylbenzocaine and N-Formylprocaine as Signature Compounds

In order to establish that the N-formyl derivatives of benzocaine and procaine were useful as signature compounds, it was necessary to demonstrate their presence in discolored authentic samples and their absence in samples that exhibited no discoloration. A number of authentic samples were analyzed for the presence of N-formylbenzocaine and N-formylprocaine. These authentic samples had been subjected to ambient and accelerated studies and exhibited either no discoloration or marked browning. Table V illustrates these results. It is evident from Table V that the N-formyl derivatives are present in those samples that discolored significantly, yet could not be detected in those samples that did not discolor. These findings supported their value as signature compounds.

* * * * *

Table V - Evaluation of N-Formyl Derivatives of Benzocaine and Procaine as Signature Compounds

Sample Number	Study	Significant Discoloration	Presence of N-Formylbenzocaine	Presence of N-Formylprocaine
19	Accelerated	Yes	-	Yes
10	Accelerated	Yes	-	Yes
49	Accelerated	Yes	-	Yes
54	Accelerated	Yes	-	Yes
18	Accelerated	Yes	-	Yes
28	Accelerated	Yes	-	Yes
54	Ambient	No	No	-
59	Ambient	No	No	-
23	Accelerated	Yes	-	Yes
47	Accelerated	Yes	-	Yes
64	Accelerated	No	No	-
65	Ambient	No	No	-
52	Accelerated	Yes	-	Yes
50	Ambient	Yes	-	Yes
22	Ambient	Yes	-	Yes

* * * * *

In order to further correlate the presence of N-formyl derivatives with the browning process, the following was done. Authentic samples composed of 10% 5-HMF, 10% acid catalyst (boric acid), and 80% of either procaine HCl or benzocaine were prepared and then placed in the dark at room temperature for one day. After this time period, the samples were analyzed for the appropriate signature compounds. The N-formyl derivatives of both benzocaine and procaine were detected in these samples. Additionally, the samples exhibited marked discoloration. These results indicate that 5-HMF plays a role in the acid-catalyzed formation of the N-formyl derivatives. This is not surprising, in that 5-HMF has been associated with sugar decomposition. Furthermore, the subsequent decomposition of 5-HMF to formic acid has been reported [7,9]. Formic acid then reacts readily with either benzocaine or procaine to form the N-formyl derivatives.

Some preliminary work has been done in the isolation of additional signature compounds in an illicit sample known to have undergone discoloration. The sample consisted, in part, of cocaine HCl, boric acid, benzocaine, and dextrose. The N-formylbenzocaine signature was detected in this sample. The additional signature compounds were colored substances and isolated as described below. The discolored sample was placed on a dilute HCl - Celite 545 AW column and eluted with water-saturated ethyl ether

as described earlier for benzocaine-containing samples. After discarding the ether eluate, the column was eluted with water-saturated chloroform. The chloroform was evaporated to less than 1 cc and transferred to the top of a neutral alumina column. The column was eluted initially with diethyl ether followed by ether-chloroform mixtures and then finally chloroform. A series of purple, blue, and pink-colored compounds eluted through the column (note: Rodd's Chemistry of Carbon Compounds states that furfural reacts with primary amines, resulting in a ring opening and condensation with the amine to form a red-colored compound). Analysis of the combined column eluates on silica gel TLC plates revealed the presence of a number of colored substances (blue, purple, pink, and yellow). Though these colored compounds appear promising as signatures, further work must be done for their full characterization.

Summary

The study described in this paper can be summarized as follows:

A.   The illicit and authentic samples that underwent discoloration, or browning, contained a primary aromatic amine, namely procaine or benzocaine, and a sugar, either lactose or dextrose; the samples also contained an acid catalyst, such as cocaine HCl, boric acid, benzoic acid, etc.

B.   The rate of discoloration was pH dependant, i.e., the rate of sample browning increased as the pH of the sample decreased.

C.   The rate of sample discoloration increased as the temperature of the sample increased.

D.   N-Formyl derivatives of benzocaine and procaine were established as *bona fide* signature compounds in discolored samples. These derivatives are believed to be formed following the decomposition of 5-HMF to formic acid and the subsequent formylation of the aromatic amine function in benzocaine and procaine.

E.   The presence of colored compounds (yellow, blue, purple, and pink) in the discolored samples appear promising as additional signature compounds.

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