J Forensic Sci, Nov. 2005, Vol. 50, No. 6
Paper ID JFS2005121
Available online at: www.astm.org

TECHNICAL NOTE

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Alkaloid Content of the Seeds from *Erythroxylum Coca* var. *Coca*

ABSTRACT: Alkaloid extracts from the seeds of *Erythroxylum Coca* var. *Coca* grown in the Chapare Valley of Bolivia were subjected to gas and liquid chromatographic-mass spectrometric analyses. Several alkaloids from these seeds were detected and characterized, including methylecgonidine, tropine, 3α-acetoxytropane, ecgonine methyl ester, cuscohygrine, N-norbenzoyltropine, benzoyltropine, hexanoylecgonine methyl ester, cocaine, *cis*-cinnamoylcocaine, and *trans*-cinnamoylcocaine. Methylecgonidine was determined to be the primary constituent and not an analytical artifact. Additionally, two significant new uncharacterized alkaloids were established as present. Recent evidence suggests that some cocaine processors are adding this seed extraction material to cocaine extracted from coca leaf and may impact cocaine impurity signature profiles.

KEYWORDS: forensic science, mass spectrometry, cocaine, tropane alkaloids, coca seeds

Recent debriefings of coca growers and cocaine base producers in the Chapare Valley of Bolivia reveal that some growers/processors are extracting coca seeds for their alkaloid content. The extraction of these alkaloids is a by-product of seed preparation for planting new coca fields. Coca seeds mature from a green berry to a red mature fruit that is about the size of a green pea. The seeds must be soaked in water to loosen a thin pulp from the encapsulated seed. Once loosened, the seed is separated from the pulp and sun-dried prior to planting new fields. The encapsulated seed fruit is commonly referred to as the seed.

Processors have discovered that they can isolate alkaloids from the seed preparation remnants. This recovered material is then added to cocaine base that has been extracted from coca leaf. The illicit method for extracting cocaine from coca leaf has been well documented (1). The same general illicit procedure is now utilized for isolating alkaloids from coca seeds. Seeds are collected by picking from the coca plant. Seeds are most plentiful on plants that are at least three years old. Usually, immature and mature seeds are simultaneously picked. Once the seeds have been soaked for 48 h (about 50 lb. of seed in 180 L of water for example), they are rolled in the hands to remove any unloosened pulp and set aside. The water solution is filtered, lime (about 60 g) and approximately 20 L of diesel fuel are added to the filtrate, and the resulting solution is mixed well. The layers are allowed to separate and the diesel layer is then transferred to a bucket containing approximately 10 L of dilute sulfuric acid. The two layers are mixed well to back extract the alkaloids into the dilute acid, afterwhich the layers are allowed to separate. The diesel is then removed. Ammonium hydroxide is added to the dilute acid to precipitate the alkaloids, which are then captured by filtration through a cloth. The dried material is white in appearance. This material is added to cocaine base that has been similarly isolated from coca leaf to increase its weight and afford a greater profit to the seller. One processor claimed he/she obtained 200 g of this material from 50 lb. of coca seeds.

There are several reports in the literature concerning the alkaloid content of coca seeds and their initial reproductive tissues prior to seed development (2–7). In those reports, methylecgonidine, hygrine, tropinone, ecgonine methyl ester, cuscohygrine, tropacocaine, cocaine, *cis*-cinnamolycocaine, and *trans*-cinnamolycocaine were detected. One report failed to detect alkaloids in the seeds (8). If some processors are adding this material to cocaine base, it may have a significant impact on the impurity signature profile(s) utilized in many forensic laboratories today. In this study, coca seeds from the Chapare Valley of Bolivia were obtained and determined for their alkaloid profile through analytical and instrumental methods.

Experimental

Plant Material

Coca seeds were collected from *Erythroxylum Coca var. Coca* (*E. coca* v. *coca*) plants during June 2004 in the Chapare Valley of Bolivia. The seeds were sun dried prior to transportation to our laboratory located in the United States. The seeds were frozen and stored at −5°C prior to workup and analysis.

Solvents, Chemicals, and Materials

All solvents were distilled-in-glass products of Burdick and Jackson Labs (Muskegon, MI). N-Methyl-N-trimethylsilyltrifluoro-acetamide (MSTFA) was obtained from Pierce Chemical (Rockford, IL). All other chemicals were of reagent-grade quality.

Standards and Precursors

3α-Acetoxytropane was synthesized from tropine and acetic anhydride, each products of Aldrich Chemical (Milwaukee, MI). All

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Received 9 March 2005; and in revised form 20 May 2005; accepted 4 June 2004; published 14 Sept. 2005.

other alkaloid standards and their spectra were acquired from the authentic reference collection of this laboratory.

Gas Chromatography/Mass Spectrometry (GC/MS)

GC/MS analyses were performed using an Agilent Model 5973 quadrupole mass-selective detector (MSD) interfaced with an Agilent Model 6890 gas chromatograph. The MSD was operated in the electron ionization mode (EI) with an ionization potential of 70 eV, a scan range of 34–700 mass units, and at 1.34 scans/s. The GC system was fitted with a 30 m × 0.25 mm ID fused-silica capillary column coated with DB-1 (0.25 μm) (J & W Scientific, Rancho Cordova, CA). The oven 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) and a temperature of 280°C. The auxiliary transfer line to the MSD was operated at 280°C.

Liquid Chromatography/Mass Spectrometry (LC/MS)

Chromatography was performed using a Waters 2525 HPLC pump fitted with a 150 mm × 4.60 mm C-18 Phenomenex column. The flow was optimized at 1.0 mL/min, using the following reversed-phase gradient: (A) water containing 0.1% trifluoroacetic acid and (B) acetonitrile. The gradient profile started at 95% A, changed linerarly to 75% A and 25% B in 15 min, held 5 min, ramped to 5% A and 95% B linearly in 5 min, and finally returned to 95% A and 5% B in 1 min. The HPLC eluent was introduced into a Waters Micromass ZQ single quadrupole mass spectrometer using Atmospheric Pressure Chemical Ionization (APCI) with positive ion detection. The detector operated in the scan range of 150–700 mass units, a scan time of 0.5 sec, and an inter-scan delay of 0.1 sec.

Isolation of Alkaloids from Erythroxylum Coca var. Coca Seeds

The following procedure is representative of the isolation from either immature seeds or mature seeds. Coca seeds were macerated in a blender with approximately three times their weight of 0.18 *M* H_2SO_4, transferred to a large beaker, and allowed to soak for 2 h with intermittent stirring. The mixture was filtered via suction filtration and the seed pulp cake was rinsed further with its equal weight of water. The filtrate was adjusted to pH 9 with solid sodium carbonate and extracted with 3 × 200 mL of methylene chloride.

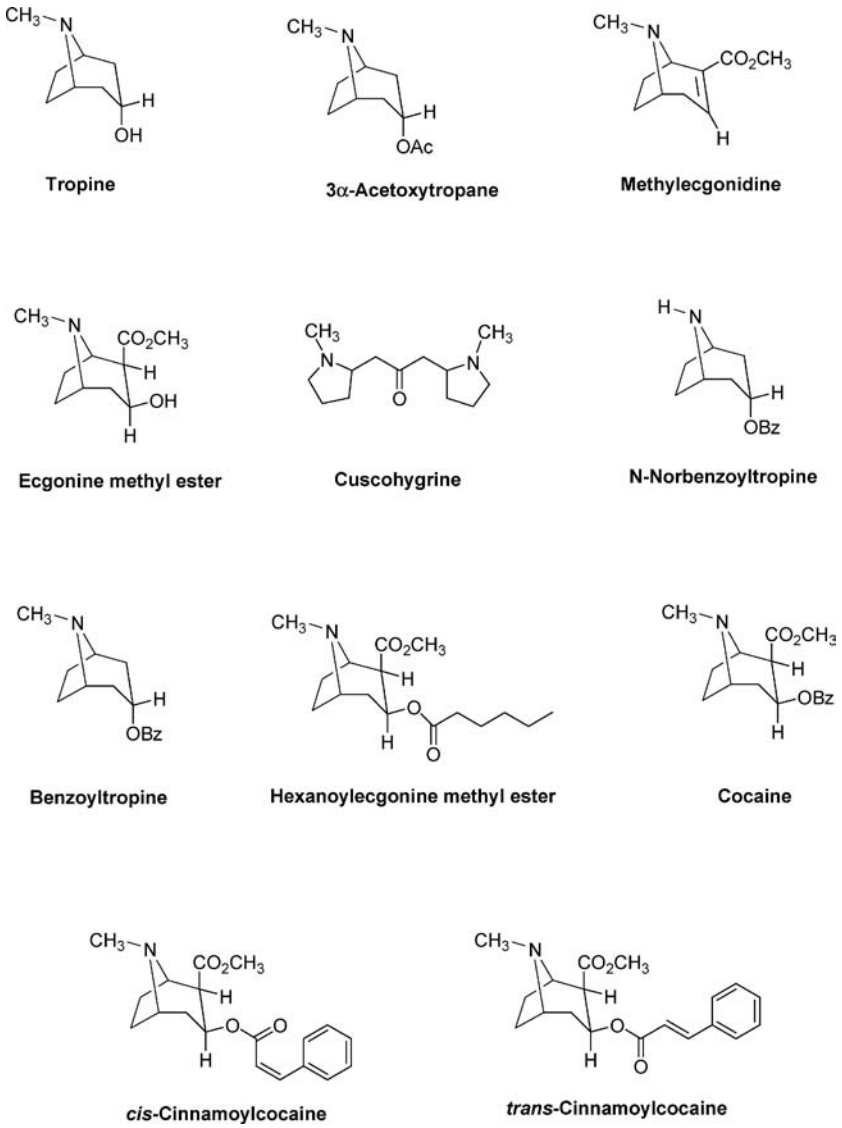

FIG. 1—*Structural formulas of alkaloids found in the seeds of E. coca v. coca.*

The combined extracts were dried over anhydrous sodium sulfate, filtered and evaporated *in vacuo* to a residue. Crude alkaloid recoveries for mature seeds (218.4 g) and immature seeds (527.0 g) were 0.104 g and 0.455 g, respectively. Total alkaloid content for these seeds was found to be <0.1%. Portions of the recovered alkaloids were then subjected to GC-MS analysis both underivatized and derivatized with MSTFA. LC-MS analyses were also performed as described.

Results and Discussion

This work reports the detection and characterization of several alkaloids (Fig. 1) from *E. Coca v. Coca* seeds via a combination of EI-GC/MS and APCI-LC/MS, in addition to comparison of known authentic and synthetic compounds to the targeted compounds.

As illustrated in Fig. 2, a peak rich total ion chromatogram (TIC) was obtained by GC/MS analysis. Many of the uncharacterized peaks are thought to be N-methylpyrrolidine alkaloids related to hygrine and cuscohygrine. This class of compounds are characterized by the presence of an EI-MS base peak at m/z 84, that typically represents the N-methylpyrrolidinium ion (9,10).

Peaks #1, #2, and #7 (minor components) were characterized as tropine, 3α-acetoxytropane, and benzoyltropine, respectively. The relative abundances of the ions at m/z 82 and m/z 83 for each were consistent with a 3α-oxo substituent (11). The mass spectrum of peak #2 has an apparent molecular ion at m/z 183 and a base peak at m/z 124, as illustrated in Fig. 3*a*. The presence of m/z 124 is indicative of a 3-oxo substituted tropane. The molecule ion difference of 42 from tropine suggested that the compound contained an acetoxy substituent. Comparison of the mass spectrum and retention time of peak #2 to synthesized standards of 3α- and 3β-acetoxytropane confirmed that it was 3α-acetoxytropane. 3α-Acetoxytropane has not been previously reported in *E. Coca v. Coca*.

Peaks #3 and #4 were characterized as methyl ecgonidine and ecgonine methyl ester, respectively. Methylecgonidine, sometimes referred to as anhydroecgonine methyl ester (peak #3), was found to be the predominant alkaloid, followed by ecgonine methyl ester (peak #4); each was confirmed by its mass spectrum. Methylecgonidine was reported as the only constituent over 60 years ago using non-spectrometric methods (2). Each can be produced as an analytical artifact from the elimination of benzoic acid and one mole of water from cocaine (12) and/or from truxilline degradation (13) in a GC injection port. However, LC-MS analysis of the extracts confirmed the *bona fide* presence of methylecgonidine and ecgonine methyl ester, giving early eluting peaks yielding a $[M + H]^+$ at m/z 182 consistent with the molecular weight of 181 and $[M + H]^+$ at m/z 200 consistent with the molecular weight of 199, respectively. Also, LC-MS analysis of the extracts indicated only an ultra-trace level of one truxilline isomer, thus confirming both compounds as being non-artifactual.

Peak #5 was identified as cuscohygrine from its mass spectrum (14) and retention time. Although it was isolated in this work, it does not precipitate from solution due to its high solubility in aqueous solutions. Therefore, it is not expected to be present to any appreciable extent in the product obtained from the illicit isolation process.

The mass spectrum of peak #6 (minor component) yields a molecular ion of m/z 231 and a base peak of m/z 110, as illustrated in Fig. 3*b*. The presence of m/z 110 is indicative of an N-nor-3-oxo substituted tropane. The molecular ion difference of 14 from tropacocaine and benzoyltropine suggested that the compound is the N-nor derivative of one of these compounds. Comparison of the mass spectrum and retention time of peak #6 to authentic standards confirmed that it was the 3α-isomer, N-norbenzoyltropine. N-norbenzoyltropine has not been previously reported in *E. Coca v. Coca*.

The mass spectrum of peak #8 (minor component) yields a molecular ion of m/z 297 and a base peak of m/z 182. The presence of m/z 182 and m/z 198 indicates a 2-carbomethoxy-3-oxo substitution. Peak #8 was identified as hexanoylecgonine methyl ester,

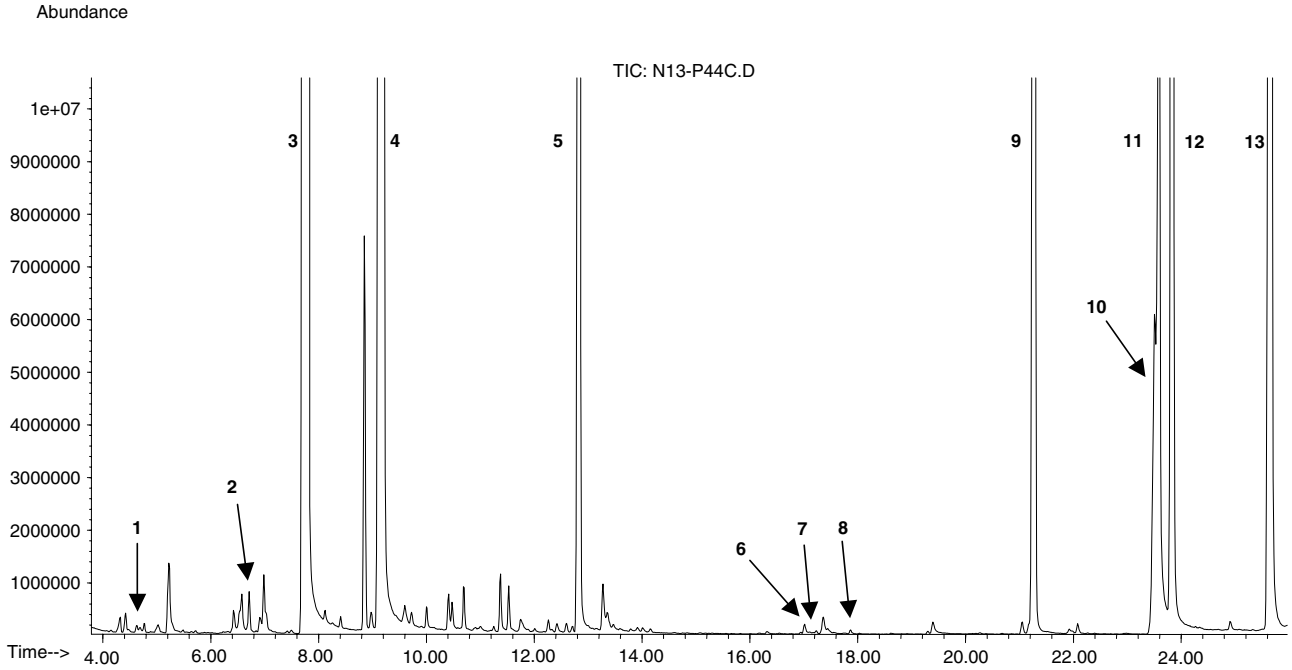

FIG. 2—*Partial reconstructed total ion chromatogram of the coca seed extract. Peaks: 1 = tropine, 2 = 3α-acetoxytropane, 3 = methylecgonidine, 4 = ecgonine methyl ester, 5 = cuscohygrine, 6 = N-norbenzoyltropine, 7 = benzoyltropine, 8 = hexanoylecgonine methyl ester, 9 = cocaine, 10 = uncharacterized nortropane, 11 = uncharacterized tropane, 12 =* cis*-cinnamoylcocaine, and 13 =* trans*-cinnamoylcocaine.*

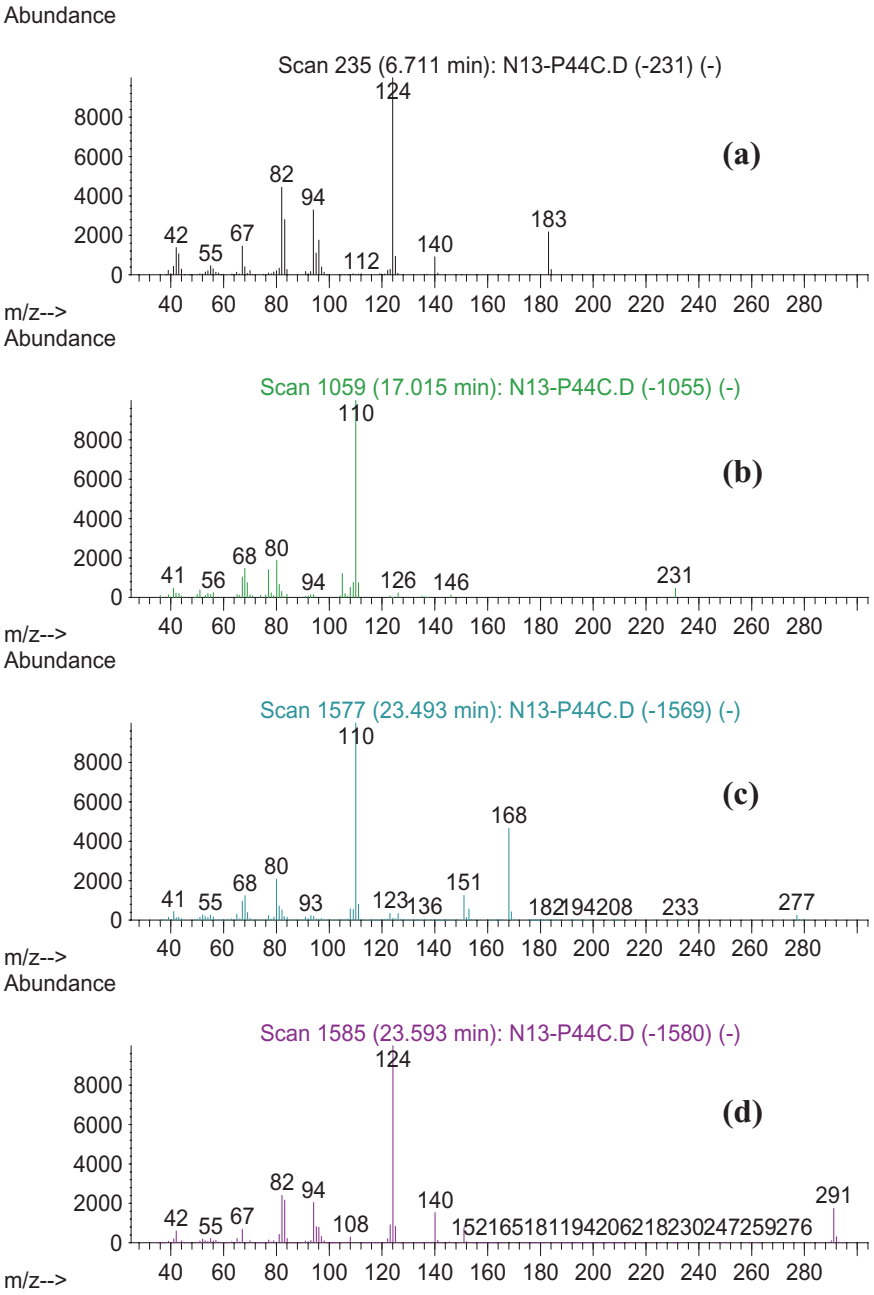

FIG. 3—*Electron ionization mass spectra of (a) 3α-acetoxytropane, (b) N-norbenzoyltropine, (c) uncharacterized nortropane, and (d) uncharacterized tropane.*

from comparison to its known mass spectrum (15) and retention time.

Peaks #9, #12, and #13 were identified as cocaine, *cis*-cinnamoylcocaine, and *trans*-cinnamoylcocaine, respectively. The relative abundances of the individual cinnamoyl-cocaines was roughly equal to that of cocaine in the seed extract. This is in contrast to cinnamoylcocaine/cocaine ratios found in *E. coca* v. *coca* leaf (9), where average *cis*- and *trans*-cinnamoylcocaine content relative to cocaine is 18% and 22%, respectively.

Peaks #10 and #11 were not baseline resolved, and constituted approximately 5% of the total ion current. Each gave a mass spectrum that suggests the two alkaloids are structurally related, yet they have not been previously encountered in our work or reported by others. Peak #10 yields a molecular ion at m/z 277 and a base peak at m/z 110, as illustrated in Fig. 3*c*. The presence of m/z 110 can be attributed to an N-nor-3-oxo substituted tropane. The presence of m/z 151 suggests elimination of water from a parent ion of m/z 168. Upon timethylsilylation with MSTFA, the base peak and molecular ion shift to m/z 182 and m/z 421, respectively. These shifts suggest that two labile protons are present on the molecule, one on the nitrogen and one possibly on the C-2 or C-3 functional group. LC-MS analysis of the extracts confirmed a molecular weight of 277, yielding a $[M + H]^+$ at m/z 278. This alkaloid (peak #10) remains uncharacterized.

The mass spectrum of peak #11 yields a molecular ion of m/z 291 and a base peak of m/z 124, as illustrated in Fig. 3*d*. The base peak of m/z 124 can be attributed to either a 3-oxo substituted tropane or a 2-carboxy-3-oxo substituted tropane. The relative abundances of m/z 82 vs. m/z 83 indicate that a 3-oxo substitution is in the α position. The presence of a minor ion at m/z 151 again suggests

elimination of water from a minor parent ion of m/z 168. Upon timethylsilylation with MSTFA, the base peak remains unchanged while the molecular ion shifts to m/z 363. This shift suggests that only one labile proton is present on the molecule. It is unclear if this proton is attributed to a carboxylic acid or hydroxyl moiety. LC-MS analysis of the extracts confirmed a molecular weight of 291, yielding a $[M + H]^+$ at 292. This alkaloid (peak #11) also remains uncharacterized until we acquire more botanical materials for future isolation and structural elucidation.

Conclusions

Eleven alkaloids were identified in the seeds of *E. coca* v. *coca*. Additionally, two major tropane alkaloids in these seeds were also found, but remain uncharacterized. Mass spectra are presented to aid forensic chemists that may encounter these compounds in illicitly produced cocaine that has been adulterated with alkaloid extracts from coca seeds. Illicit cocaine produced from coca leaf, adulterated with coca seed extracts, may give unusual analytical signature profiles with elevated levels of methylecgonidine, ecgonine methyl ester, and cinnamoylcocaines, as well as the two new uncharacterized tropane alkaloids. Future work is anticipated in acquiring more coca seeds, thus enabling us to isolate the two unknown compounds for further characterization.

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