TECHNICAL NOTE

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Detection and Determination of Pseudococaine in Coca Leaves and Illicit Cocaine Samples

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ABSTRACT: Methodology is presented for the isolation, identification and determination of pseudococaine in coca leaves and illicit cocaine. Coca leaves, crude cocaine base (coca paste), refined cocaine base and refined cocaine hydrochloride, all derived from the same geographic location in Bolivia, were examined. Pseudococaine and other coca alkaloids were isolated from leaf samples using toluene extraction followed by acid/Celite trap and ion-pair column chromatography, and from crude and refined cocaine samples by acid/Celite column ion-pairing chromatography. Mass spectral analysis of coca leaf isolates confirmed the presence of pseudococaine. Pseudococaine was quantified by capillary gas chromatography with flame ionization detection at levels of 0.0001–0.035% (relative to cocaine) in refined illicit cocaine and coca leaves.

KEYWORDS: criminalistics, cocaine, pseudococaine, coca leaves, chromatographic analyses, illicit drugs

Within the past several years considerable attention has focused on the identification and determination of impurities in illicit cocaine. Many of these impurities are by-products resulting from the crude manufacturing processes, while others are naturally occurring alkaloids carried through the extraction and crystallization procedures to the refined illicit product. Forensic laboratories have developed numerous chromatographic methods for the determination of these impurities for comparative purposes [*1*].

Pseudococaine and cocaine, as illustrated in Fig. 1, are C-2 equatorial and axial epimers, respectively. Pseudococaine was originally identified in coca leaves over a hundred years ago [*2*], but its presence was later attributed as an artifact from the methodology work-up [*3*]. Although pseudoecgonine, a pseudococaine hydrolysis product, has been previously identified in illicit cocaine [*4*], pseudococaine had been only tentatively identified to date [*5*]. Recent work in our laboratory has definitively characterized pseudococaine in coca leaf and illicit cocaine samples.

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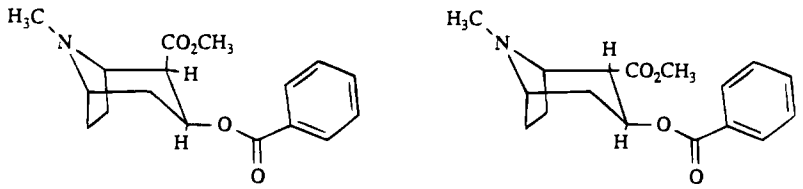

FIG. 1—*Structures of pseudococaine and cocaine.*

In this study, pseudococaine was isolated from both coca leaf and illicit cocaine through modifications of our previously reported isolation procedures for trace coca alkaloids [*6,7*]. Pseudococaine was determined in the isolates by gas chromatography with flame ionization detection (GC-FID) and confirmed via comparison of its mass spectra with a known standard. The analysis of a pseudococaine-free cocaine standard using the methodology described herein did not produce pseudococaine as a method artifact.

Experimental

Coca Leaf and Illicit Cocaine

The leaves were collected from the Chapare Valley in Bolivia and were identified as Erythroxylum coca var. coca. Leaves were sun/air-dried to constant weight, stored over silica gel and were less than four months old at the time of analyses. A crude cocaine base (coca paste) sample and refined cocaine base sample were produced from these same leaves via illicit manufacturing processes [*8*]. Five additional refined cocaine base samples from other Chapare grown leaves were also obtained. Finally, an amount of a refined cocaine base was converted to illicit cocaine hydrochloride also via illicit manufacturing process.

Solvents, Standards and Materials

All solvents were "distilled-in-glass products" of Burdick and Jackson Labs (Muskegon, MI). Dextromethorphan, used as the internal standard (ISTD), was obtained from Sigma Chemical (St. Louis, MO). Pseudococaine was obtained from the reference drug collection of this laboratory and pharmaceutical-grade cocaine hydrochloride from a commercial source. All solvents used for column chromatography were water-saturated and were prepared as previously described [*6*].

Gas Chromatography

A Hewlett-Packard Model 5890 Series II gas chromatograph was used to generate all standard and sample chromatograms. A 30 m × 0.25 mm i.d. fused-silica capillary column coated with DB-1701 (J & W Scientific) at a film thickness of 0.25 μm was employed. Hydrogen (99.999%, UHP) was the carrier gas and the inlet was pressure programmed to maintain a constant linear velocity of 35 cm/s. The injection port and flame ionization detector were maintained at 230°C and 300°C, respectively. Samples were injected in the split mode (20:1). The oven temperature was programmed as follows: initial temperature, 170°C; initial hold, 1.0 min; program rate, 6.0°C/min; final temperature, 275°C; final hold, 17.5 min. All chromatograms were recorded at an attenuation of 2 unless otherwise noted.

Mass Spectrometry

Mass spectra were obtained on a Hewlett Packard Model 5971A Mass Selective Detector (MSD) interfaced with a Hewlett Packard 5890 Series II gas chromatograph. The MSD

operated under electron ionization (E1) conditions at 70 eV and in full scan mode. The GC oven temperature parameters were identical to those utilized for the GC-FID analyses, except that the final temperature and hold were 285°C and 11.0 min, respectively.

Extraction and Isolation of Pseudococaine and Other Alkaloids from Powdered Coca-Leaf

The preparative isolation of pseudococaine was accomplished using a modification of recently developed methodology [*6,7*]. To each of four 200 mL centrifuge tubes was added 10 g powdered leaves (containing 0.70 % cocaine) and 10 mL of saturated aqueous sodium bicarbonate. After trituration, 400 mL of H_2O-saturated toluene, containing 100 μg of dextromethorphan internal standard, was added to the tubes (ca 100 mL/tube). The tubes were heated at 65–70°C for 1 h with mixing every 15 min. After centrifugation, the toluene extracts were transferred to a flask. The coca-leaf powder was extracted again with 75 mL of H_2O-saturated toluene in each tube in the manner just described. The toluene extracts were combined and transferred to a chromatographic column (600 × 45 mm) packed with a mixture of 25 mL of 0.36 N H_2SO_4 and 50 g of Celite 545. After discarding the eluate, an additional 150 mL of H_2O-saturated toluene followed by 150 mL of H_2O-saturated $CHCl_3$ were added to the column, the eluates also being discarded. The alkaloids were then liberated from the column by elution with 150 mL of water-saturated $CHCl_3$ containing 1.5 mL of diethylamine, followed by 200 mL of H_2O-saturated $CHCl_3$. The eluate was extracted with 500 mL of pH 4.0 acid phthalate buffer and the buffer was then back-extracted with three 25-mL aliquots of $CHCl_3$. The combined $CHCl_3$ extracts were dried over Na_2SO_4 and evaporated *in vacuo* to an oily residue.

This residue was reconstituted in approximately 500 μL of H_2O-saturated $CHCl_3$ and transferred to a column (260 × 22 mm) packed with: bottom layer—a mixture of 1 g Celite 545 and 0.5 mL saturated aqueous sodium bicarbonate; top layer—a mixture of 4 g Celite 545 and 2.0 mL of 2 M NaCl/1 N HCl solution. Approximately 25 mL of H_2O-saturated $CHCl_3$ was added to the column and the first 10 mL collected. This eluate was concentrated under a stream of nitrogen to approximately 1 mL and submitted to GC and GC-MS analyses. A duplicate batch of leaves was similarly processed.

Isolation of Pseudococaine from Crude and Refined Cocaine Samples

The isolation of pseudococaine from cocaine samples was accomplished using a modification of recently developed methodology [*6*]. A 100 mg equivalent of cocaine base was weighed into a 13 mL centrifuge tube followed by 0.50 mL of H_2O-saturated $CHCl_3$, containing 5 μg of ISTD, and 0.5 mL saturated aqueous sodium bicarbonate. After vigorous mixing, the $CHCl_3$ phase was removed, triturated with 0.5 g of Celite 545 and packed into a Celite column (260 × 22 mm) containing: bottom layer—a mixture of 1 g Celite 545 and 0.5 mL saturated aqueous sodium bicarbonate; top layer—mixture of 4 g Celite 545 and 2.0 mL of 2 M NaCl/1 N HCl solution. The column was eluted with H_2O-saturated $CHCl_3$; the first 10 mL of eluate were collected and evaporated to ca 100 μL for cGC-FID analyses.

Determination of Pseudococaine

Approximately 2 μL from the coca leaf, cocaine base and cocaine HCl isolates were injected in duplicate into the gas chromatograph under the conditions described. Quantitative data was obtained using two standard mixtures in $CHCl_3$. Standard #1: pseudococaine—0.200 mg/mL and dextromethorphan—0.100 mg/mL. Standard #2: pseudococaine—0.020 mg/mL and dextromethorphan—0.100 mg/mL.

Results and Discussion

Ion-Pair Chromatography

The primary function of ion-pair chromatography (IPC) was the isolation of pseudococaine from the bulk cocaine matrix. Cocaine is retained by the column while many other alkaloids, including pseudococaine, ion-pair under the conditions used [*6,7*]. Dextromethorphan was selected as the internal standard because it also ion-pairs well under these conditions. A study revealed that both pseudococaine and dextromethorphan were recovered in the ion-pair isolates from the columns at levels >98%.

Method Artifacts

The formation of method artifacts must be considered when identifying and determining trace levels of alkaloids in natural products. Artifacts may be produced both from the interaction of chemicals and/or solvents with cocaine and other alkaloids during sample preparation/extraction or as a result of gas chromatographic-induced degradation. The generation of anhydroecgonine methyl ester [*9,10,11*], ecgonine methyl ester [*11*] and pseudoecgonine methyl ester [*11*] as analytical artifacts have all been previously documented. It should be noted that pseudococaine was *not* produced as an analytical artifact from the rigorous experimental conditions in the study in which pseudoecgonine methyl ester was identified. Although pseudococaine has been conveniently prepared from cocaine in a two step synthesis [*12,13*], direct formation of pseudococaine from cocaine is inhibited due to simultaneous hydrolysis of the C-3 benzoyloxy ester, generating pseudoecgonine methyl ester.

Pharmaceutical grade cocaine, devoid of pseudococaine, was used as a method blank to determine whether the methodology created pseudococaine as an artifact. Approximately 40 g of coca leaves, which had been exhaustively extracted for removal of coca alkaloids, was spiked with an equivalent amount of pseudococaine-free, pharmaceutical-grade cocaine. When the leaves were processed in the same manner as described previously, no pseudococaine was detected.

Structural Characterization of Pseudococaine in Coca Leaf

The presence of pseudococaine in coca leaf was confirmed by its mass spectrum. The GC retention time and mass spectrum of the leaf extract were virtually identical to that of synthesized pseudococaine standard. Although pseudococaine and cocaine are diastereoisomers and have very similar spectra, they can be differentiated by the relative intensities of ions observed at m/z 94, 96 and 152, as illustrated in Fig. 2.

Pseudococaine Content of Coca Leaf and Illicit Cocaine

The quantitative results for Bolivian coca leaf, crude cocaine base, refined cocaine base and refined cocaine hydrochloride are presented in Table 1. Figure 3 illustrates the chromatography of a typical refined cocaine base sample. Though not seen in Fig. 3, later eluting peaks (15 to 34 min) in the chromatogram were present. The concentration of pseudococaine was greatest in the leaf samples. Duplicate analyses of the leaves gave only a minor variation in results (0.034% vs. 0.036%). Analysis of crude cocaine base (coca paste) derived from those same leaves revealed a 3X decrease in pseudococaine (0.013%). The refined cocaine base sample #2 (Table 1) derived from the aforementioned crude base #1 (Table 1) revealed a 20X and 10X decline in pseudococaine concentration relative to the leaves and crude cocaine base, respectively. The diminished presence of pseudococaine in a refined base sample by an order of magnitude is most probably due to the conditions employed during the purification process. The values for pseudococaine in the remaining

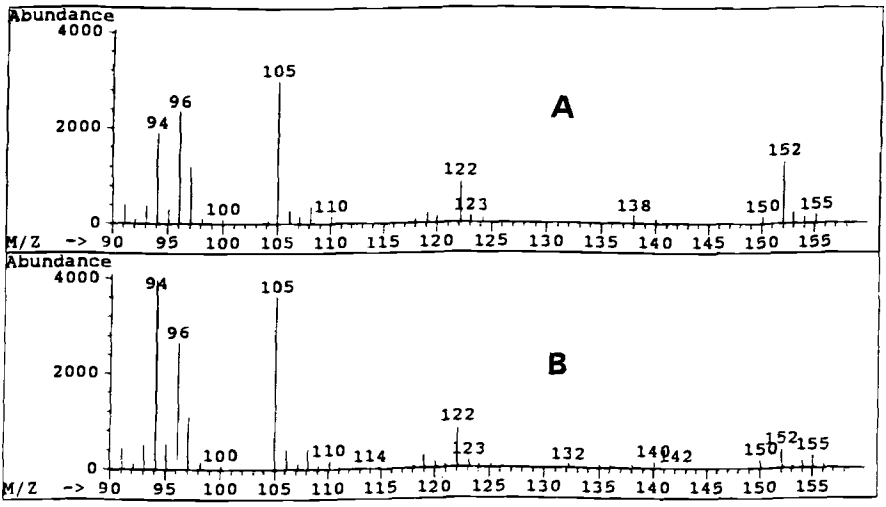

FIG. 2—*Vertical expansion (90–160 amu) of electron ionization mass spectra. A = Pseudococaine and B = Cocaine.*

TABLE 1—*Quantitative results for pseudococaine in coca leaves and illicit cocaine.*

Sample[a]	Pseudococaine, %[b]
Coca leaves	0.035
Crude cocaine base (#1)[c]	0.013
Refined cocaine base (#2)[d]	0.0014
Refined cocaine base (#3)	0.010
Refined cocaine base (#4)	0.0025
Refined cocaine base (#5)	0.0025
Refined cocaine base (#6)	0.0096
Refined cocaine base (#7)	0.0043
Refined cocaine HCl (#8)[e]	<0.001

[a]Samples obtained from the Chapare Valley of Bolivia.
[b]Pseudococaine results are % w/w and are calculated relative to cocaine content.
[c]Produced from above coca leaves.
[d]Produced from crude cocaine base #1.
[e]Produced from refined base #6.

five refined cocaine base samples ranged from 0.0025 to 0.010%. These samples were each derived from separate lots of Chapare Valley-grown coca leaf. The refined cocaine hydrochloride sample, which was derived from refined base sample #6 (Table 1), exhibited a 10X reduction in pseudococaine. It can be seen in Table 1 that the concentration of pseudococaine decreased approximately 50X, relative to cocaine, during the complete coca leaf-to-cocaine hydrochloride manufacturing process.

Conclusions

Methodology has been presented for the determination of pseudococaine in coca leaves, crude cocaine base (coca paste), refined cocaine base and refined cocaine hydrochloride. Quantitation was accomplished using capillary gas chromatography-flame ionization detec-

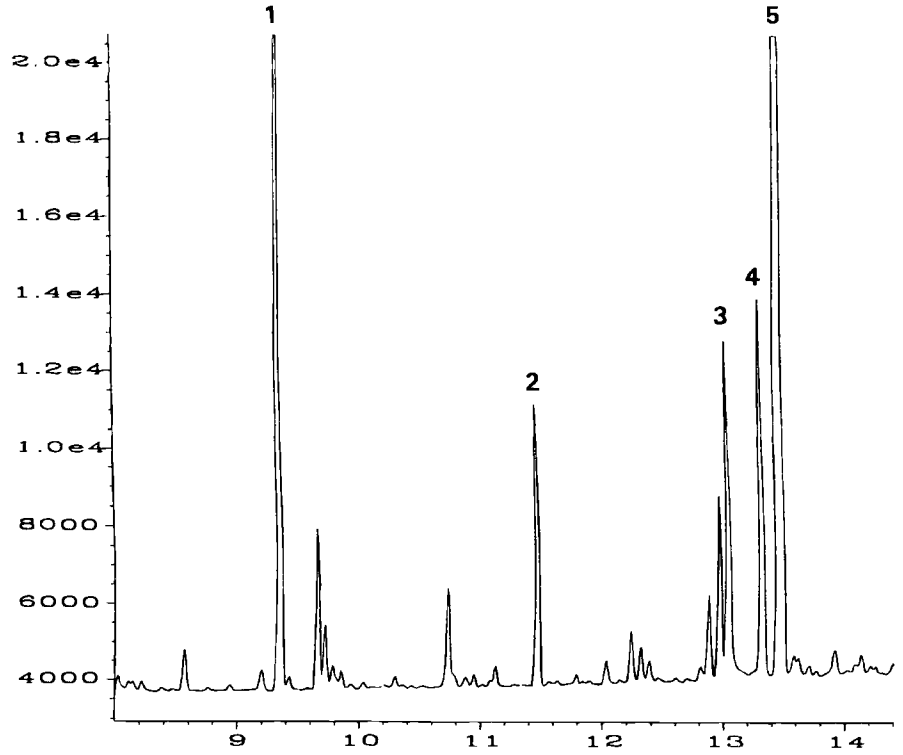

FIG. 3—*Capillary gas chromatogram of an ion-pair eluate from an illicit refined cocaine base exhibit containing pseudococaine. Peak identification (min)—1 = tropacocaine (9.3 min), 2 = dextromethorphan internal standard (11.5 min), 3 = norcocaine (13.0 min), 4 = pseudococaine (13.3 min), 5 = cocaine (13.5 min).*

tion after isolation from the bulk matrices. Pseudococaine was definitively identified using mass spectrometry by comparison to a synthesized standard.

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