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Determination and in-depth chromatographic analyses of alkaloids in South American and greenhouse-cultivated coca leaves

James M. Moore*, John F. Casale, Robert F.X. Klein and Donald A. Cooper

United States Drug Enforcement Administration, Special Testing and Research Laboratory, 7704 Old Springhouse Road, McLean, VA 22102-3494 (USA)

John Lydon

United States Department of Agriculture, Agricultural Research Services, Weed Science Laboratory, Building 001, Room 236, Beltsville, MD 20705 (USA)

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ABSTRACT

Methodology is described for the detection and/or determination of cocaine and minor alkaloids in South American coca as well as in greenhouse- and tropical-cultivated field coca of known taxonomy. Coca leaf from Bolivia, Peru, Ecuador and Colombia were subjected to the determination of cocaine, *cis*- and *trans*-cinnamoylcocaine, tropacocaine, hygrine, cuscohygrine and the isomeric truxillines. The greenhouse samples were cocaine-bearing leaves of the genus *Erythroxylum* and included *E. coca* var. *coca*, *E. novogranatense* var. *novogranatense* and *E. novogranatense* var. *truxillense*, and the alkaloids determined were cocaine, ecgonine methyl ester, cuscohygrine, tropacocaine and the cinnamoylcocaines. The tropical-cultivated coca were *E. novogranatense* var. *novogranatense* and *E. coca* var. *coca*. Cocaine and minor alkaloids were isolated from basified powdered leaf samples using a toluene extractant, followed by acid–Celite column chromatography. The isolated alkaloids were determined by capillary gas chromatography with flame ionization or electron-capture detection. Methodology is also presented for the isolation and mass spectral analysis of numerous trace-level coca alkaloids of unknown structure.

INTRODUCTION

It is well-recognized in forensic drug research that the characterization of manufacturing impurities and byproducts present in illicit drugs is useful for intelligence purposes. This may include (a) ascertaining the geographic origin of the drug, (b) doing chemical comparative analyses to determine if different drug seizures are related and derived from a common source and (c) evaluation of the clandestine manufacturing processes for precursor chemical monitoring. The manufacturing impurities and byproducts most studied and reported have been those associated with cocaine, heroin and the amphetamine-type compounds [1–20].

Although most illicit drugs are produced via chemical syntheses, some are derived directly from natural products. Such an example is illicit cocaine, a drug which is isolated from the leaves of the South American coca plant, *Erythroxylum* (*E.*) *coca* var. *coca*, using multiple extraction and purification steps. There have been an increasing number of publications describing the in-depth gas chromatographic (GC) analyses of manufac-

* Corresponding author.

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turing impurities/byproducts in refined illicit cocaine seizures [1,12–34]. However, scant attention has been paid by forensic chemists to the in-depth analysis of *E. coca* var. *coca* or other cocaine-bearing plants, or how their alkaloidal content relates to impurities and byproducts present in refined illicit cocaine.

A review of the literature indicated that virtually all of the quantitative data for coca-leaf alkaloids have been provided for only cocaine and the cinnamoylcocaines [35–44]. Therefore, methodology was developed and described herein that utilizes toluene extraction and column chromatography for the isolation of coca alkaloids from the leaf, followed by their determination using capillary GC (cGC)–flame ionization detection (FID) or cGC–electron-capture detection (ECD). This methodology is suitable for the determination of cocaine and most other known alkaloids in cocaine-bearing plants, including *cis*- and *trans*-cinnamoylcocaine, the isomeric truxillines, tropacocaine, ecgonine methyl ester, cuscohygrine and hygrine. This study encompasses the analyses of coca leaf samples from the field in South America, *i.e. E. coca* var. *coca* (ECVC-SA) and *E. novogranatense* var. *novogranatense* (ENVN-SA), as well as greenhouse-cultivated *E. coca* var. *coca* (ECVC-GH), *E. novogranatense* var. *novogranatense* (ENVN-GH) and *E. novogranatense* var. *truxillense* (ENVT-GH). Tropical-cultivated samples, grown at a site other than South America, included *E. novogranatense* var. *novogranatense* (ENVN-TR) and *E. coca* var. *coca* (ECVC-TR).

Methodology is also described for the isolation of numerous unknown trace-level alkaloids from leaf tissue and the bulk cocaine matrix of South American coca. This was accomplished largely by trap, ion-pairing and liquid–liquid partition column chromatography and acid buffer extractions. Mass spectral data were acquired for about 100 of these suspected alkaloids.

EXPERIMENTAL

Plant material

All leaf material examined in this study contained significant levels of cocaine. While several varieties of cocaine-producing *Erythroxylum* are known, cocaine-bearing leaves are sometimes referred to collectively as coca leaves, regardless of the species/variety of the plant from which they were harvested. Unless referring to a specific species, we use that terminology herein. Leaves analyzed in this study included collections from the field in Bolivia, Peru, Ecuador and Colombia. Other leaves were harvested from coca plants of three different varieties cultivated in a greenhouse environment at the US Department of Agriculture. Also included in this study were leaves from plants cultivated at a tropical site other than South America.

Two batches of leaves originating from Bolivia/Peru and leaves from Colombia were believed to be ECVC-SA and ENVN-SA, respectively. ECVC-SA leaves are the most commonly associated with the clandestine manufacture of illicit refined cocaine. Leaves harvested in Ecuador were of unknown taxonomy. These South American field samples were believed harvested from plants cultivated by local natives. After harvesting, the leaves were sun/air-dried and then sent to our laboratory for analyses. The dried leaves were stored at room temperature for 1–3 years prior to in-depth alkaloid analyses.

Plants grown in a greenhouse at the US Department of Agriculture in Beltsville, MD, USA were ECVC-GH, ENVN-GH and ENVT-GH. Greenhouse cultivars were grown in 1.2–l pots containing seven parts greenhouse potting media (sandy loam) and three parts Promix B1 (Premier Brands, New York, NY, USA) (pH 6.0, 4.1% organic matter). Plants were watered to soil saturation as needed and fertilized with 2.5 g/l of N–P–K–(20:20:20) fertilizer every third week. The plants were approximately 2 years old at the time of harvest. Leaves were air-dried at room temperature for two days and then stored over silica gel at 0°C.

Cultivated at a non-South American tropical site were ENVN-TR and ECVC-TR. These plants were of the same seed stock used in the greenhouse cultivar of the same species (ENVN-GH and ECVC-GH). The soil at the tropical site location was a Halii gravelly sandy loam [classified as Typic Gibbshumox (an oxisol)] containing 6% organic matter (pH 5.4). The site was at an

elevation of 170 m and received a mean annual rainfall of 250 cm. Plants did not receive supplemental watering and were fertilized with 30 kg/ha N–P–K (20:20:20) per month. Plants were about 1.5 years old at time of harvest. Leaves were air-dried at room temperature for 2 days and then stored over silica gel at 0°C.

Solvents, chemicals, standards and materials

All solvents were distilled-in-glass products of Burdick & Jackson Labs. (Muskegon, MI, USA). Heptafluorobutyric anhydride (HFBA) and N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA), both supplied in 1-ml sealed glass ampules, were obtained from Pierce (Rockford, IL, USA). A 1.0 *M* solution of lithium aluminum hydride ($LiAlH_4$) in diethyl ether and 4-dimethylaminopyridine (DMAP) were supplied by Aldrich (Milwaukee, WI, USA). Cocaethylene, used as methodology internal standard, was prepared by the benzoylation of ecgonine ethyl ester. Hygrine, ethylhygrine, cuscohygrine, ecgonine methyl ester, ecgonine ethyl ester and *trans*-cinnamoylcocaine were synthesized in our laboratory. Dimethyl-μ-truxinate was prepared as described in ref. 18. Pharmaceutical-grade cocaine base and tropacocaine·HCl were obtained from commercial sources. The Celite 545 stationary phase used for column chromatography was obtained from J.T. Baker (Jackson, TN, USA) and was used without any pre-treatment. A pH 4.0 acid phthalate buffer was prepared according to the United States Pharmacopeia XIX [48]. All other chemicals were of reagent-grade quality.

Gas chromatography

Three gas chromatographs were used in this study. All used hydrogen as carrier gas at 30–40 cm/s. A Hewlett-Packard 5890 Series II GC–FID system, operating in a split mode of 20:1 and fitted with a 30 m × 0.25 mm I.D. fused-silica capillary column coated with DB-1 (0.25 μm) (J & W Scientific, Rancho Cordova, CA, USA), was used for the concomitant determination of ecgonine methyl ester, cuscohygrine, tropacocaine, cocaine and *cis*-/*trans*-cinnamoylcocaine in the greenhouse- and tropical-cultivated coca. The oven temperature was programmed as follows: initial temperature, 150°C; initial hold, 1.0 min; program rate, 6.0°C; final temperature, 275°C; final hold, 8.0 min. Injector and detector temperatures of 230 and 280°C, respectively, were maintained. Nitrogen was used as auxiliary gas. A Perkin-Elmer Sigma 2000 GC–FID system was utilized for the analysis of South American-cultivated coca, under conditions similar to those described above.

A Hewlett-Packard 5880A GC–^{63}Ni ECD system, operating in the splitless mode and fitted with two capillary columns, was used for the analyses of the isomeric truxillines and hygrine. Argon–methane (95:5) was used as auxillary gas. Chromatographic conditions for the truxillines analyses are given in refs. 18 and 25. Hygrine was chromatographed on a 15 m × 0.25 mm fused-silica capillary column coated with DB-5 + (0.25 μm). The oven temperature for the hygrine quantitation was programmed as follows: (level 1) initial temperature, 90°C; initial hold, 5.5 min; program rate, 5°C/min; final temperature, 160°C; final hold, 5 min; (level 2) program rate, 4°C/min; final temperature, 275°C; final hold, 10 min. Injector and detector temperatures were both held at 300°C.

Mass spectrometry (MS)

A Hewlett-Packard Model 5971 quadrupole mass-selective detector interfaced with a Hewlett-Packard 5890 Series II gas chromatograph was used in this study. The mass-selective detector was operated in the electron impact ionization mode with an ionization potential of 70 eV, a secondary electron multiplier value of 1541 and at 1.2 scans/s. The GC system was fitted with a 30 m × 0.25 mm I.D. fused-silica capillary column coated with DB-1 (0.25 μm). The oven temperature was programmed as follows: initial temperature, 150°C; initial hold, 1.0 min; program rate, 6.0°C/min; final temperature, 285°C; final time, 11 min. Injector and detector temperatures were maintained at 230 and 280°C, respectively.

Extraction and isolation of coca-leaf alkaloids

Air-dried leaf tissue was powdered with a Wiley mill to pass a 2-mm sieve. If not used immediately, the powder was placed in a screw-

capped glass jar and stored over silica gel at about 0°C.

About 1 g of coca-leaf powder was accurately weighed into a 50-ml centrifuge tube and triturated with 1 ml of an aqueous solution saturated with sodium hydrogencarbonate. To this basified mixture were added 20 ml of water-saturated toluene containing 2.00 mg of cocaethylene internal standard. The tube was heated at 60–65°C for 1 h with occasional mixing. After centrifugation (*ca.* 400 *g*) for 10 min, the toluene supernatant was transferred to a flask. The coca-leaf powder residue in the tube was then extracted with two additional 15-ml aliquots of water-saturated toluene as just described. The three toluene extracts were combined, mixed thoroughly and transferred to a chromatographic column (260 mm × 22 mm) packed with a mixture of 2.0 ml of 0.18 *M* sulfuric acid and 4 g of Celite 545. After passing through the column, the toluene eluate was discarded. An additional 20 ml of water-saturated toluene followed by 20 ml of water-saturated $CHCl_3$ were then added to the column and the eluates discarded. The coca alkaloids were then liberated from the column by the addition of 50 ml of water-saturated $CHCl_3$ containing 250 μl of diethylamine. After adjustment to 50.0 ml, the eluate containing the coca alkaloids, referred to as solution A, was mixed thoroughly with 10 g of anhydrous Na_2SO_4 and saved for alkaloid quantitation (note: for coca-leaf samples with lower alkaloid content, greater sample masses and/or a concentration step may be required).

GC quantitation of isolated alkaloids

Ecgonine methyl ester, cuscohygrine, tropacocaine, cocaine and cis/trans-cinnamoylcocaine.

About 2–3 μl of solution A from above was injected in duplicate into the 30-m DB-1 capillary column under chromatographic conditions described above for the Hewlett-Packard 5890 Series II GC–FID system. Quantitative data were obtained using a standard mixture of target alkaloids in 50 ml of chloroform, at the following concentrations: cocaine 0.100 mg/ml, cocaethylene internal standard 0.040 mg/ml, cuscohygrine 0.050 mg/ml, ecgonine methyl ester 0.050 mg/ml and *trans*-cinnamoylcocaine 0.030 mg/ml. The 50 ml of standard solution also contained about 100 μl of diethylamine. Calculations for *cis*-cinnamoylcocaine in samples were based upon peak area for the standard *trans*-cinnamoylcocaine.

If review of a sample chromatogram revealed only trace levels of certain alkaloids, especially tropacocaine, an appropriate aliquot of solution A was reduced in volume (5–10 ×), but not to dryness, *in vacuo* at 45–50°C and then reinjected into the 30-m DB-1 capillary column as described previously.

Truxillines. For determination of the truxillines, an appropriate aliquot (1.0–5.0 ml) from solution A was transferred to a centrifuge tube, containing 5.0–25.0 μg of dimethyl-μ-truxinate internal standard, and evaporated to dryness. To the residue were added 250 μl of chloroform and the tube heated at 75°C for 3 min with occasional vortex mixing. The residue in the tube was then subjected to $LiAlH_4$ reduction followed by HFBA derivatization and cGC–ECD analysis as described in ref. 18. All quantitative calculations for the isomeric truxillines used the dimethyl-μ-truxinate internal standard as reference compound. After using a conversion factor, all results were ultimately reported as % (w/w) truxillines dry relative to the cocaine content of the leaf [25].

Hygrine. For the determination of hygrine, recently developed methodology was used [45]. To a 5.0–10.0-ml aliquot from solution A was added an accurate quantity (approximating suspected levels of hygrine in sample) of ethylhygrine internal standard and the solution extracted with 10–20 ml of a pH 4.0 acid phthalate buffer, discarding the chloroform phase. The buffer phase was made basic by the addition of sodium hydrogencarbonate and then extracted with several small aliquots of chloroform, filtering each extract through anhydrous sodium sulfate. To the combined chloroform extracts was added a minimum amount of HCl and the chloroform evaporated to dryness. The residue was treated with a minimum volume of chloroform followed by reduction with 1 *M* $LiAlH_4$ in diethyl ether and derivatization with HFBA in the presence of DMAP. Quantitative analysis was accomplished by cGC–ECD using a 15-m DB-5 capillary

column under conditions described above. Quantitative data were obtained using a standard mixture of known amounts of hygrine and ethylhygrine internal standard that were subjected to $LiAlH_4$ reduction and HFBA/DMAP derivatization.

Isolation of trace unknown alkaloids from coca leaves

For the mass spectral analyses of trace levels [< 1% (w/w) relative to cocaine] of suspected alkaloids in the coca leaf matrix, 150–200 g of Peruvian/Bolivian powdered coca leaf was used. Extraction and column chromatographic isolation of the alkaloids were accomplished as described previously, using toluene as extractant and a dilute sulfuric acid–Celite chromatographic column, except material masses and volumes were scaled up to accommodate the increased sample mass. The final Celite column chloroform–alkaloid eluate was dried over sodium sulfate and then reduced in volume under vacuum (50°C) to a syrupy residue.

The residue was reconstituted in 50–100 ml of chloroform and extracted with twice its volume of pH 4.0 acid phthalate buffer. The buffer was back-extracted with two aliquots of chloroform, which were combined with the original chloroform extract and set aside as fraction A. The buffer was made basic with sodium hydrogencarbonate and extracted with several small aliquots of chloroform. The combined chloroform extracts were passed through a glass column (600 mm × 40 mm) packed with a mixture of 25 ml of pH 4.0 acid phthalate buffer and 50 g Celite 545. The eluate was dried over sodium sulfate and then reduced under vacuum (at *ca.* 50°C) to a small volume, identified as fraction B.

The pH 4.0 buffer–Celite column above was eluted with sufficient diethylamine in water-saturated chloroform to liberate any retained alkaloids. The eluate was dried over sodium sulfate and then reduced under vacuum to a small volume, identified as fraction C.

Fraction A from above was passed through a glass column (600 mm × 40 mm) packed with a mixture of 50–75 ml of 1 *M* HCl–1 *M* NaCl and 100–150 g of Celite 545. The eluate was monitored in 10-ml fractions until 100 ml had been collected, which were subsequently identified as fractions D_{1-10}.

The 1 *M* HCl–1 *M* NaCl–Celite column from above was eluted with sufficient diethylamine in water-saturated chloroform to liberate any retained alkaloids. The eluate was dried over sodium sulfate and then reduced under vacuum (40–50°C) to a small volume, identified as fraction E.

Aliquots from each of fractions A–E were evaporated to dryness and the residues treated with 500 μl of chloroform–MSTFA (1:1) at 75°C for 30 min. The derivatized samples were subjected to cGC–MS analyses under conditions already described.

RESULTS AND DISCUSSION

Before a discussion of results, it is prudent to recognize that only four South American coca leaf samples, three greenhouse specimens and two non-South American tropical-cultivated leaf samples were subjected to quantitative analyses in this study. Since it is believed that the alkaloid content of the coca leaf may vary upon a number of factors including the age of the plant/leaf, soil composition, altitude of cultivation, soil composition, longitudinal location, environmental factors and the time lapse between leaf harvesting and analysis, the quantitative results reported herein should be viewed from that perspective. With the analysis of many more samples, the aforementioned factors will play a less significant role in the evaluations of results.

Method accuracy and reproducibility

The quantitative results for the ENVN-GH coca leaf obtained by the method described herein were compared with data obtained using a modification of a direct extraction/dilution procedure. The modification of this latter method [41] involved the quantitative extraction of the alkaloids from 1.500 g of ENVN-GH coca leaf with warm methanol and dilution to volume with chloroform that contained cocaethylene internal standard and diethylamine. The comparative quantitative data for these two methods are given in Table I. Fig. 1 illustrates the alkaloid chromatography for the ENVN-GH leaf using

TABLE I

COMPARATIVE DETERMINATION OF ALKALOIDS IN *E. NOVOGRANTENSE* VAR. *NOVOGRANATENSE* USING TOLUENE *VERSUS* METHANOL EXTRACTION

All quantitative data are reported as the average of duplicate analyses; all chromatographic determinations were the average of duplicate injections. Cocaine quantitative results are reported as % (w/w) relative to air-dried leaf; all other alkaloids are reported as % (w/w) relative to cocaine content. The toluene extraction method is as described in this paper in the Experimental section. The methanol extraction method was a modification of a procedure reported by Solon and Sperling [41].

Alkaloid	Methodology	
	Toluene extraction	Methanol extraction
Cocaine	0.38	0.36
Ecgonine methyl ester	63	?[a]
Cuscohygrine	11	?[b]
Tropacocaine	4.6	?[a]
cis-Cinnamoylcocaine	50	51
trans-Cinnamoylcocaine	170	165

[a] Unable to quantify because of peak interference by compound from coca leaf extract.

[b] Unable to quantify because of absence of cuscohygrine peak, perhaps caused by degradation during extraction or co-adsorption with coca leaf extract in cGC system.

the method described herein. As seen in Table I, good agreement was found between the two methods for cocaine and *cis*- and *trans*-cinnamoylcocaine content. However, peaks 3 (cuscohygrine) and 5 (unknown hydroxy-substituted alkaloid), seen in Fig. 1, were not present in the chromatogram of the direct dilution [41] method, due probably to some suppression phenomenon. One possibility is irreversible adsorption of these two polar compounds, along with plant components, in the GC–FID injection port. Furthermore, peaks 2 (ecgonine methyl ester) and 4 (tropacocaine) in Fig. 1 could not be determined from the chromatogram of the direct dilution method because of coeluting impurities attributed to non-alkaloidal coca leaf compounds.

As seen in Table I, the direct dilution method using methanol was less than satisfactory for the

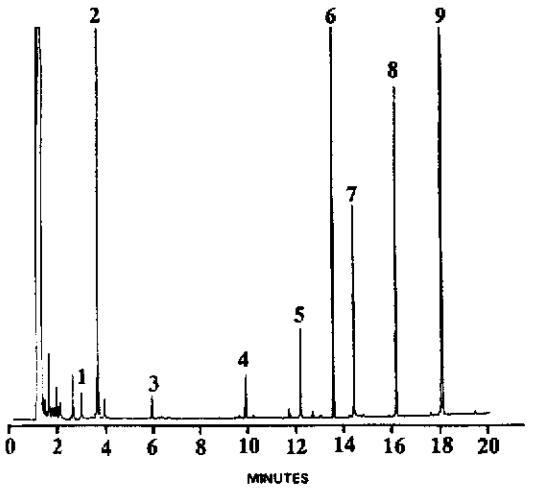

Fig. 1. The cGC–FID chromatogram of major coca alkaloids isolated and determined in greenhouse-cultivated *E. novograntense* var. *novograntense*. Peaks: 1 = ecgonidine methyl ester (2.9 min); 2 = ecgonine methyl ester (3.6 min); 3 = cuscohygrine (5.8 min); 4 = tropacocaine (9.8 min); 5 = unidentified coca alkaloid (12.1 min); 6 = cocaine (13.5 min); 7 = cocaethylene internal standard (14.3 min); 8 = *cis*-cinnamoylcocaine (16.1 min); 9 = *trans*- cinnamoylcocaine (18.0 min).

in-depth analysis of alkaloids in coca leaf. This is evidenced by the non-detection of alkaloids represented by peaks 3 and 5 in Fig. 1. Furthermore, the direct injection of coca leaf extracts into the GC system resulted in interfering peaks due to plant components. Finally, there was evidence that suggested repeated injections of plant extracts into the GC system caused a degradation in column performance.

The efficacy of the truxillines determination referenced herein has been previously determined [18,25]. The accuracy of that methodology was notably enhanced by using the structurally related dimethyl-μ-truxinate as internal standard. Similarly, the hygrine determination employed a structurally related internal standard, namely, ethylhygrine, to improve accuracy. However, as will be discussed subsequently, there was some question as to whether the reported presence of hygrine was due, in part or wholly, to the degradation of cuscohygrine.

The ENVN-GH leaf sample (see Fig. 1) was also subjected to a quantitative reproducibility study for selected alkaloids. A total of seven

sample analyses was accomplished over the period of one week. A sample mass of 1.5 g of coca leaf powder was used for each determination, with a final dilution of 50 ml. The reproducibility results, reported in Table II, were determined to be acceptable for all target alkaloids. As seen, the most reproducible results were for cocaine and *cis*- and *trans*-cinnamoylcocaine. This was not surprising, given their higher levels in coca leaf and the fact that a structurally related internal standard, cocaethylene, was used in their analyses. The data for cuscohygrine may be a reflection of its instability, non-homogeneity in leaf tissue and/or its higher vapor pressure, which may result in losses during the basification and mixing of the coca leaf powder. The results in Table II, as well as in Table I, indicated that when using a sample mass of 1.5 g and a final dilution of 50 ml, the minimum % (w/w) (relative to cocaine) for acceptable reproducibility/accuracy for cuscohygrine and tropacocaine was about 5–10% and 2–4%, respectively. Concentrations below these required a concentration step and/or a greater sample mass.

Alkaloid content of greenhouse- and tropical-cultivated coca leaf

The quantitative results for ECVC-GH, ENVT-GH, ENVN-GH, ENVN-TR and ECVC-TR are found in Table III. In Fig. 1 is the chromatogram used for the determination of alkaloids in the ENVN-GH coca leaf sample. In all cases, a sample mass of 1.000 g and a dilution of 50.0 ml were used. For some samples the inclusion of a concentration step was necessary to enhance chromatographic response and achieve good accuracy for cuscohygrine and tropacocaine. Cocaethylene was used as internal standard for all samples and in the standard mixture.

All compounds listed in Table III, excepting cuscohygrine, are tropane alkaloids. Cuscohygrine, an N-methylpyrrolidine alkaloid, was at significantly higher levels in the two ECVC samples than in either ENVN or ENVT varieties. Conversely, the tropacocaine and *cis*- and *trans*-cinnamoylcocaine content in the ECVC samples was markedly below that of the other varieties. As will be seen, these findings also pertained to South American samples.

Ecgonine methyl ester, when present in illicit refined cocaine samples, may be the result of cocaine hydrolysis during clandestine cocaine manufacture. However, the methodology herein did not produce significant levels of this compound and, therefore, was considered a *bona fide* coca leaf alkaloid. Peak 1 in Fig. 1 was identified as ecgonidine methyl ester, believed to be formed as a result of the thermal degradation of the isomeric truxillines in the GC injection port [18]. The presence of this artifact was more pronounced in samples with higher truxillines content, especially ENVN and ENVT. Because

TABLE II

REPRODUCIBILITY OF QUANTITATIVE RESULTS FOR ALKALOIDS IN GREENHOUSE-CULTIVATED *E. NOVOGRANATENSE* VAR. *NOVOGRANTENSE*

A coca powder sample mass of 1.500 g was used for each analysis, with a final dilution volume of 50 ml. Quantitative result for cocaine was the average of 7 analyses and reported as % (w/w), relative to air-dried coca leaf. Quantitative results for alkaloids other than cocaine were the average of 7 analyses and reported as % (w/w), relative to the cocaine content. All ± results are standard deviations; $n = 7$.

Cocaine	Ecgonine methyl ester	Cuscohygrine	Tropacocaine	Cinnamoylcocaine	
				cis	*trans*
0.371 ± 0.004	62.8 ± 3.4	10.8 ± 1.1	4.60 ± 0.26	50.1 ± 0.8	170.5 ± 1.6

TABLE III

QUANTITATIVE RESULTS FOR COCAINE AND OTHER COCA ALKALOIDS IN GREENHOUSE- AND TROPICAL-CULTIVATED COCA LEAVES

All cocaine results are % (w/w) and are calculated relative to dry leaf mass. Results for all alkaloids, excepting cocaine, are % (w/w) and are calculated relative to cocaine content.

Alkaloid	ECVC-GH[a]	ENVT-GH[b]	ENVN-GH[c]	ENVN-TR[d]	ECVC-TR[e]
Cocaine	0.54	0.60	0.37	0.43	0.67
Ecgonine methyl ester	57	38	63	29	47
Cuscohygrine	57	3.8	11	5.8	61
Tropacocaine	0.3[f]	1.4	4.6	3.8	0.16
cis-Cinnamoylcocaine	7.2	25	50	53	18
trans-Cinnamoylcocaine	18	46	98	170	22

[a] Greenhouse-cultivated *E. coca* var. *coca*.
[b] Greenhouse-cultivated *E. novogranatense* var. *truxillense*.
[c] Greenhouse-cultivated *E. novogranatense* var. *novogranatense*.
[d] Tropical-cultivated *E. novogranatense* var. *novogranatense*.
[e] Tropical-cultivated *E. coca* var. *coca*.
[f] Sample also contained benzoyltropeine, an epimer of tropacocaine, at a level of 1.1% (relative to cocaine content).

of its artifactual presence, quantitative levels of ecgonidine methyl ester were not determined. Perhaps the most unexpected result in Table III was the presence of benzoyltropeine at a level 3 × greater than that for its epimer, tropacocaine (benzoyl-ψ-tropeine). This is believed to be the first report of such a level of benzoyltropeine in coca leaf.

Another compound seen in Fig. 1, but not given in Table III, was peak 5. This peak has yet to be characterized, but preliminary mass spectral analysis suggested it to be a hydroxy-substituted tropane alkaloid, having an apparent molecular mass of 261. Its presence was, by far, the greatest in ENVN-GH. It was also detected at lower levels in ENVN-TR and ENVT-GH, but not in ECVC-GH or ECVC-TR. This compound is believed to be a major new alkaloid.

The truxillines and hygrine were not determined in the greenhouse samples. Their determination in greenhouse coca leaf will be the subject of a future report.

Alkaloid content of South American coca leaf

In-depth alkaloid determinations were also provided for field samples cultivated and harvested in the countries of Peru, Bolivia, Colombia and Ecuador. In Table IV are given the results for cocaine, the cinnamoylcocaines, tropacocaine and cuscohygrine. As seen, the most disparate data belonged to the Colombian leaf, believed to be ENVN. The higher results for the cinnamoylcocaines and tropacocaine, compared to the ECVC cultivars of Peru and Bolivia, were consistent with the data in Table III for greenhouse- and tropical-cultivated coca. Of particular interest in Table IV was the ratio of the cinnamoylcocaine *trans*-to-*cis* isomers. The *trans*/*cis* ratios of the four South American field samples were markedly below that for the greenhouse cultivars in Table III. A smaller ratio was also realized for the tropical-cultivated ECVC in Table III. These results are consistent with previous postulations that the *trans* isomer of cinnamoylcocaine is the first-formed alkaloid and is partially converted to the *cis* isomer via photoisomerization [46]. Presumably, the greenhouse cultivars would not be subjected to the full array of ultraviolet radiation from sunlight as is field-cultivated coca. A higher *trans*/*cis* ratio has also been reported for growth chamber-grown ECVC [44]. An exception to the foregoing was the high *trans*/*cis* ratio for the ENVN-TR coca leaf sample.

The truxilline quantitative data for the South American coca are presented in Table V. Fig. 2

TABLE IV

QUANTITATIVE RESULTS FOR COCAINE AND OTHER COCA ALKALOIDS IN SOUTH AMERICAN-CULTIVATED COCA LEAVES

All cocaine results are % (w/w) and are calculated relative to dry leaf mass. Results for all alkaloids, excepting cocaine, are % (w/w) and are calculated relative to cocaine content.

Country	Cocaine	Cinnamoylcocaine		Tropacocaine	Cuscohygrine
		cis	*trans*		
Bolivia	0.70	8.6	6.0	0.34	78
Peru	0.72	5.8	2.9	0.25	51
Ecuador	0.36	6.6	7.4	1.6	11
Colombia	0.44	28	33	4.9	33

illustrates the chromatography of the truxilline alkaloids present in the Peruvian coca leaf. As seen in Table V the lowest truxilline values were associated with the coca leaf from Peru and Bolivia. These results were consistent with truxilline levels found in illicit refined cocaine hydrochloride seized in those countries [47]. Relative to cocaine, the total truxilline content for the Ecuadoran leaf was 4–5 × higher than that from Peru/Bolivia. The Colombian sample had the highest truxilline levels, the total being more than 60% of its cocaine content. A review of the individual truxilline ratios for the four samples revealed no remarkable differences in their intra-sample truxilline ratios. Such data would only be meaningful, however, if derived from a much larger data base.

In Table VI are the quantitative results for hygrine in South American coca. Fig. 3 illustrates the chromatography of hygrine and ethylhygrine internal standard in the Colombian coca leaf. Both hygrine (peaks 1 and 2) and ethylhygrine (peaks 3 and 4) internal standard are each represented by two peaks. These are the di-HFB derivatives of diastereomeric pairs. These diastereomers are created upon $LiAlH_4$ reduction of hygrine and ethylhygrine to yield hygrinol and ethylhygrinol, respectively [45].

TABLE V

ISOMERIC TRUXILLINE CONTENT OF SOUTH AMERICAN-CULTIVATED COCA LEAVES

All isomeric truxilline results calculated using the μ-isomer as reference standard. All data presented as % (w/w) relative to cocaine.

Truxilline	Bolivia	Peru	Ecuador	Colombia
α-	0.74	0.87	3.51	20.4
β-	0.62	0.74	3.22	14.5
δ-	0.46	0.50	1.82	9.2
ϵ-	0.30	0.35	1.36	6.2
ω-	0.11	0.15	0.84	2.8
γ-	0.11	0.15	0.64	2.5
neo-	0.09	0.11	0.61	2.4
peri-	0.05	0.05	0.33	1.4
ζ-	0.03	0.05	0.50	1.2
epi-	0.02	0.02	0.21	0.64
Total	2.53	2.99	13.04	61.2

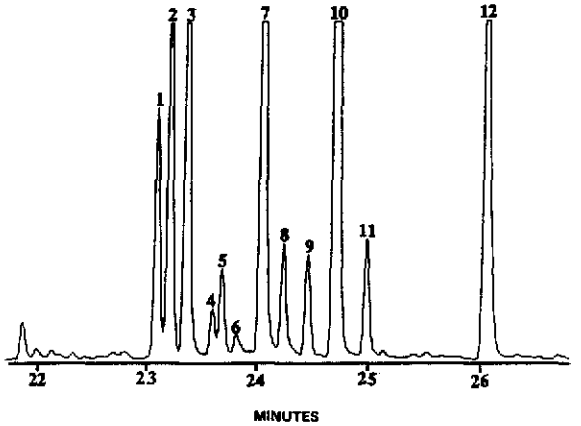

Fig. 2. The cGC–ECD chromatogram of the isomeric truxillines determined in Peruvian coca leaves. Pertinent chromatographic peaks are the result of $LiAlH_4$ reduction of the truxilline followed by derivatization with heptafluorobutyric anhydride to yield a di-O-HFB derivative. Peaks (truxilline isomers): 1 = ϵ- (23.00 min); 2 = δ- (23.21 min); 3 = β- (23.37 min); 4 = *peri*- (23.60 min); 5 = *neo*- (23.69); 6 = *epi*- (23.82 min); 7 = α- (24.06 min); 8 = ω- (24.24 min); 9 = γ- (24.46 min); 10 = μ- internal standard (24.72 min); 11 = ζ- (24.94 min); 12 = heneicosanol internal standard (26.06 min).

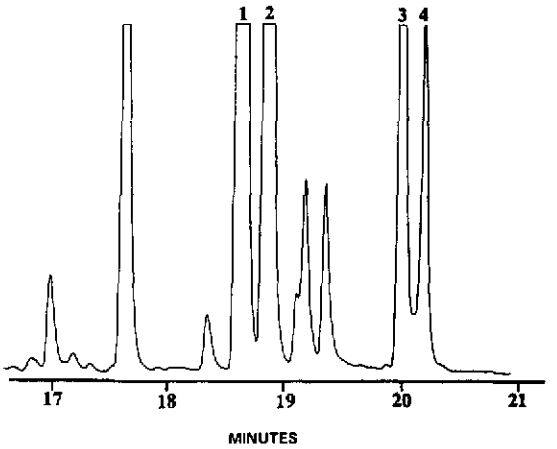

Fig. 3. The cGC–ECD chromatogram of hygrine determined in Colombian coca leaves. Peaks: 1, 2 (18.6 min, 18.8 min) = di-HFB derivatives of hygrinol diastereomers (obtained via $LiAlH_4$ reduction and HFBA derivatization of hygrine); 3, 4 (20.0 min and 20.2 min) = di-HFB derivatives of ethylhygrinol (obtained via $LiAlH_4$ reduction and HFBA derivatization of ethylhygrine internal standard).

These two alcohols each have two asymmetric carbon atoms, accounting for two diastereomeric pairs. The peak areas for each pair were summed for quantitative calculations. Though not seen in Fig. 3, later eluting peaks (28–32 min) in the chromatogram were believed due to the isomeric truxillines.

As seen in Table VI, coca leaf from Peru and Bolivia had the lowest levels of hygrine. In fact, at these low levels the question is raised as to whether these values reflect the true leaf content for hygrine or whether this alkaloid is produced wholly, or in part, from the degradation of cuscohygrine. We have previously observed that, when stored at room temperature for prolonged periods, some powdered coca samples experienced diminution in cuscohygrine content with concomitant increases in the levels of hygrine [47]. In contrast, and unexpectedly, the hygrine content of the Colombian leaf was nearly 25% of the cocaine content. Although it was not believed that this result was an anomaly, the analysis of additional samples will be required for confirmation of this finding.

TABLE VI

HYGRINE CONTENT OF SOUTH AMERICAN-CULTIVATED COCA LEAVES

Results reported as % (w/w) relative to cocaine content of air-dried leaf.

Hygrine (%)			
Bolivia	Peru	Ecuador	Colombia
2.3	1.4	4.2	24

Uncharacterized trace level coca alkaloids

For the detection and characterization of trace-level coca alkaloids, a greater quantity (150–200 g) of basified South American coca leaf powder was subjected to toluene extraction as described under Experimental. Subsequent column chromatographic and buffer extraction work-up yielded fractions A–E (see Experimental). Each fraction was subjected to trimethylsilylation with chloroform–MSTFA and then subjected to cGC–electron impact MS analyses.

In fraction A were cocaine and the bulk of the major coca alkaloids in chloroform. Fraction A

was extracted with a pH 4.0 acid phthalate buffer, which removed cuscohygrine, hygrine and numerous trace-level hydroxy-containing alkaloids. After extraction of the pH 4.0 buffer with several aliquots of chloroform (which were added to fraction A), it was basified and extracted with several more aliquots of chloroform. These extracts were subsequently passed through a pH 4.0 buffer–Celite column and the eluate concentrated to a small volume and identified as fraction B.

Fraction B contained, by far, the bulk of the unknown trace-level alkaloids amenable to cGC analysis. Over 60 trace-level compounds were detected as seen in the reconstructed total-ion chromatogram illustrated in Fig. 4. Of these compounds, about 45 yielded significant fragment ions at *m/z* 82 and 182, which suggested the presence of a carbomethoxy-substituted tropane moiety, as found in the major coca alkaloids. More than 50 suspected alkaloids contained a trimethylsilyl (TMS) moiety, as evidenced by a significant *m/z* 73 ion. It was believed that TMS attachment occurred primarily on –OH functions. These findings were are significant, because it suggested these alkaloids would also be amenable to heptafluorobutyrylation and more sensitive detection using cGC–ECD [1].

Alkaloids retained on the pH 4.0 buffer column from above were liberated using chloroform–diethylamine and the eluate reduced to a small volume which was identified as fraction C. Two major alkaloids found in this fraction were ecgonine methyl ester and cuscohygrine. Also present were 15–20 suspected alkaloids, the majority of which yielded a base-peak fragment ion at *m/z* 84, suggesting the presence of an N-methylpyrrolidine moiety, as in cuscohygrine. Also present in this fraction were compounds that gave ions at *m/z* 82, 84 and 182, indicating the presence of both N-methylpyrrolidine and carbomethoxy-substituted tropane moieties. Finally, suspected alkaloids were present that yielded a base-peak fragment ion at *m/z* 82 but did not give the usual accompanying *m/z* 182 ion. As was seen for fraction B, the majority of the alkaloids in fraction C incorporated at least one TMS group upon derivatization with chloroform–MSTFA.

Fraction A from above was subjected to ion-pairing column chromatography using 1 *M* HCl–1 *M* NaCl–Celite 545 and chloroform as eluent. A collection and examination of the ten 10-ml fractions, identified as fractions D_{1-10}, revealed that most of the trace-level alkaloids were present in the first two fractions. These early-eluting fractions contained more than 25 suspected trace-level alkaloids. More than half of these compounds yielded mass spectral fragment ions at *m/z* 82 and 182, and only about one-third contained a TMS group. Some of the TMS-substituted alkaloids gave mass spectral ions as high as 495 u. It was not known whether these high-mass ions were molecule or fragment ions.

The compounds that did not ion-pair, and were thus retained on the column from above, were liberated with chloroform–diethylamine and the eluate concentrated to a small volume, identified as fraction E. After MSTFA derivatization, this fraction was subjected to cGC–MS analysis. As expected, the reconstructed total-ion chromatogram was dominated by the presence of cocaine and the isomeric cinnamoylcocaines. Less than five suspected trace-level alkaloids were seen.

Of the more than 100 uncharacterized, trace-level alkaloids and other compounds detected in the above fractions, the possibility that some were artifactual was considered. Since many tropane alkaloids in coca leaf are diesters, their potential for hydrolysis during routine manipulations should be recognized. Furthermore, since a relatively strong organic base (diethylamine) was used in the work-up of alkaloids, the possibility of epimerization could not be discounted. Finally, the action of certain solvents upon some coca alkaloids may result in their N-demethylation. For example, the presence of trace levels of peroxides or other oxidants in some solvents can cause low-yield N-demethylation of cocaine to yield N-norcocaine [47]. After review of the mass spectra for the compounds isolated in fractions A–E from above, it was concluded that the majority of them were *bona fide* alkaloids and not artifactual. Work is ongoing in an attempt to structurally characterize and determine the levels of these 100 or so trace-level coca alkaloids.

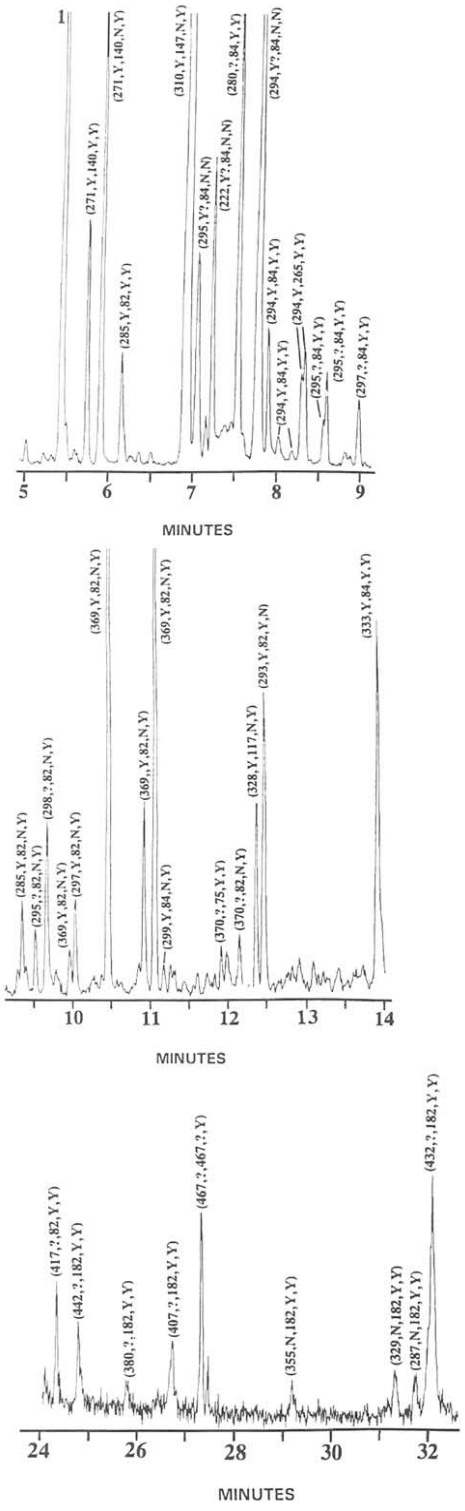

Fig. 4. The reconstructed total-ion chromatogram of suspected trace-level coca alkaloids in Peruvian/Bolivian coca leaves. Key to peak captions: (A, B, C, D, E): A = mass spectral ion of highest mass detected for peak; B = probability of high mass ion being the molecule ion, Y (yes), N (no), ? (unknown); C = mass spectral base peak; D = presence of mass spectral fragment ions m/z 82 and 182 (Y, N, ?); E = alkaloid amenable to trimethylsilalation (Y, N, ?).

CONCLUSIONS

Methodology has been presented that is suitable for the determination of cocaine, *cis*- and *trans*-cinnamoylcocaine, tropacocaine, the isomeric truxillines, ecgonine methyl ester, cuscohygrine and hygrine in South American and greenhouse-cultivated coca leaf. This included the analysis of field samples from Bolivia, Peru, Ecuador and Colombia as well as greenhouse- and tropical-cultivated coca of known taxonomy, including *E. coca* var. *coca*, *E. novogranatense* var. *novogranatense* and *E. novogranatense* var. *truxillense*. Quantitation was accomplished using cGC–FID and–ECD. Methodology was also described for the isolation and mass spectral analysis of about 100 suspected trace level alkaloids of unknown composition from Peru/Bolivian coca leaf.

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