James M. Moore,[1] *M.S. and John F. Casale,*[1] *B.S.*

Lesser Alkaloids of Cocaine-Bearing Plants. Part I: Nicotinoyl-, 2′-Pyrroloyl, and 2′- and 3′-Furanoylecgonine Methyl Ester—Isolation and Mass Spectral Characterization of Four New Alkaloids of South American *Erythroxylum coca* Var. *coca*

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ABSTRACT: Four new trace-level alkaloids of South American coca leaf, *Erythroxylum coca* var. *coca*, have been isolated from the bulk leaf/alkaloids matrix and characterized by gas chromatography-mass spectrometry and synthesis. These alkaloids, nicotinoyl-, 2′-pyrroloyl- and 2′- and 3′-furanoylecgonine methyl ester, were detected in Peruvian coca leaf and/or in a commercial, industrial-grade extract of South American coca. Alkaloid isolation methodology included toluene extraction of the leaf followed by dilute acid/Celite and alumina column chromatography and recrystallization. This methodology also allowed for the detection and partial characterization of an additional 125–150 new tropane alkaloids in coca leaf. Forensic implications are discussed.

KEYWORDS: forensic science, substance abuse, chemistry, coca leaf, cocaine, *Erythroxylum, Erythroxylum coc* var. *coca*, alkaloids, isolation, gas chromatography, mass spectrometry

Historically, the isolation and characterization of plant alkaloids has been mainly to determine their efficacy as medicinal agents. Alkaloids have also been investigated to assist in the chemotaxonomic classification of plants. Our laboratory, on the other hand, has been concerned with the characterization of alkaloids in selected plants for forensic purposes; in particular, as it applies to certain refined, illicit drugs. Although most drugs found on the illicit market do not have direct botanical origins, there are a few notable exceptions, including cocaine, morphine/heroin, and the cannabinoids.

From a law enforcement perspective, the interrelationship between alkaloids of the plant and their presence in the refined illicit drugs derived from those plants can have both strategic and tactical importance. This includes being able to evaluate chemicals and processes used in the extraction of the alkaloid from the plant and its eventual conversion to a refined drug. Thus, it is possible to monitor the diversion of chemicals and solvents to clandestine drug laboratories. Second, some illicit drugs are derived from plants, e.g., opium, that may be cultivated in disparate areas around the world. Knowledge of these plant alkaloids and their detection in the refined drug may allow for the determination of the latter's geographic origin. Third, the detection of plant alkaloids and related compounds in illicit refined drugs, especially if present at the ultratrace level, is more facile if first characterized in their botanical sources. Finally, the determination of plant alkaloids, as well as manufacturing by-products, in illicit drugs may allow for the chemical comparison of different drug seizures to determine if they came from a common source. This has been useful in the development of conspiracy cases for criminal prosecution (1).

The major source for the world's supply of illicit cocaine is the coca plant/leaf, *Erythroxylum coca* var. *coca* (*E. coca* var. *coca*), cultivated primarily in the South American countries of Peru and Bolivia, with processing to the hydrochloride carried out in these countries, but mostly in Colombia. Our laboratory has been interested for some time in the characterization of new coca alkaloids and manufacturing by-products found as impurities in illicit, refined cocaine. Furthermore, we have recently presented a study that includes the detection, structural elucidation and determination of major/minor alkaloids in South American coca and the same leaf cultivated at another tropical site and in a greenhouse (2).

Besides cocaine, earlier reports of well-known alkaloids present in the South American coca leaf have included cinnamoylcocaine, tropacocaine, ecgonine methyl ester, *alpha-* and *beta*-truxilline, hygrine and cuscohygrine (3). More recently, additional coca alkaloids have been first characterized in refined, illicit cocaine and their presence subsequently confirmed in coca leaf. These included *cis-* and *trans*-cinnamoylcocaine (4), the eleven isomeric truxillines (5,6), the hydroxycocaines (7), trimethoxy-substituted tropane alkaloids (8), and pseudococaine (9). The foregoing studies and related topics have been addressed in recent reviews (10,11).

In this first part of a new series of studies, the presence of four new trace-level alkaloids in South American coca leaf, *E. coca* var. *coca*, and/or in a commercial, industrial-grade coca leaf extract is reported. They are nicotinoyl-, 2′-pyrroloyl- and 2′- and 3′-furanolyecgonine methyl ester. Similar furanoyl isomers were previously reported in *Erythroxylum dekindtii* (12), but differed from those reported here in their configuration at C-3 and the absence of a C-2 carbomethoxy substituent. The four titled compounds were among 125–150 new trace-level alkaloids that were detected using methodology described herein. They were first isolated from

[1]Research chemist and forensic chemist, respectively, United States Drug Enforcement Administration, Special Testing and Research Laboratory, 7704 Old Springhouse Road, McLean, VA 22102-3494 (USA).

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the leaf matrix using toluene extraction, followed by trap and ion-pairing Celite column chromatography, alumina adsorption column chromatography and recrystallization techniques. All four were characterized by capillary gas chromatography-mass spectrometry and by comparison with synthesized standards.

Experimental

Plant Material and Coca-Leaf Extract

South American coca leaf, identified as *Erythroxylum coca* var. *coca* (ECVC) was obtained from cultivated fields in the Chapare Valley of Bolivia and the Upper Huallaga Valley of Peru. After harvesting, the leaf was air-dried and, just prior to analysis, powdered in a Wiley Mill to pass a 2-mm sieve. A commercial, industrial-grade South American coca leaf extract (40% cocaine), referred to as *E Harz*, was generously supplied by Stepan Chemical Co., Maywood, NJ, USA. The *E Harz* was derived from Peruvian and/or Bolivian coca leaf.

Solvents, Chemicals, and Chromatographic Materials

All solvents were distilled-in-glass products of Burdick and Jackson Labs (Muskegon, MI, USA). N-Methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA) supplied in 1-mL sealed glass ampules, was obtained from Pierce (Rockford, IL, USA). Celite 545 was obtained from Spectrum Chemical Mfg. Corp. (Gardena, CA, USA) and was used without pretreatment. A pH 4.0 acid phthalate buffer was prepared according to the United States Pharmacopeia XIX (13). All other chemicals were of reagent-grade quality.

Anhydrous neutral and basic alumina were obtained as an anhydrous product of Aldrich (Milwaukee, WI, USA) and were standard grade, Activity I, ca 150 mesh, 58 angstroms, with a surface area of 155 m^2/g. Prior to its use, water was added to the alumina to yield a moisture content of 3–4% w/w. Glass chromatographic columns were a product of Kontes (Vineland, NJ, USA).

Standards and Precursors

The precursor acid chlorides, namely, nicotinoyl chloride hydrochloride, isonicotinoyl chloride hydrochloride, and 2-furoyl (furanoyl) chloride were all products of Aldrich. Picolinoyl and 3-furoyl chloride were prepared by the reaction of picolinic acid and 3-furoic acid with an excess of sulfonyl chloride; excess sulfonyl chloride was removed by evaporating under a stream of nitrogen at 75°C.

To prepare several of the titled alkaloids and related isomers, ecgonine methyl ester hydrochloride (25–95 mg) was heated with a 50% molar excess of the appropriate acid chloride at 75°C in pyridine overnight. After removal of the pyridine under a stream of nitrogen at 70°C, the residue was dissolved in 1 mL of 1 N sulfuric acid and washed with 3 × 10-mL aliquots of ethyl ether, the latter being discarded. The solution was adjusted to pH 8–9 with an aqueous solution saturated with sodium carbonate and then extracted with chloroform. To remove unreacted ecgonine methyl ester, the chloroform extract was washed with several aliquots of a pH 5.0 acid phthalate buffer. Each chloroform extract was then dried over anhydrous sodium sulfate and finally reduced to a residue under a nitrogen stream. The standards prepared thusly were 2β-carbomethoxy-3β-nicotinoyloxytropane (nicotinoylecgonine methyl ester), 2β-carbomethoxy-3β-isonicotinoyloxytropane (isonicotinoylecgonine methyl ester), 2β-carbomethoxy-3β-picolinoyloxytropane (picolinoylecgonine methyl ester), 2β-carbomethoxy-3β-(2′-furanoyloxy)tropane (2′-furanoylecgonine methyl ester), and 2β-carbomethoxy-3β-(3′-furanoyloxy)tropane (3′-furanoylecgonine methyl ester). Yields varied from 12–78% (note: The 2α-isomers of the foregoing compounds were similarly prepared).

2′- and 3′-Pyrroloylecgonine methyl ester were prepared via reaction of their respective acid chloride with ecgonine methyl ester (and also its C-2 epimer) in the presence of dicyclohexylcarbodiimide (DCC), as previously described (8).

Capillary Gas Chromatography-Mass Spectrometry (cGC-MS)

Two cGC-MS systems were used in this study. One was a Hewlett-Packard Model 5971 quadrupole mass-selective detector (MSD) interfaced with a Hewlett-Packard 5890 Series II gas chromatograph. The MSD was operated in the electron ionization (EI) mode with an ionization potential of 70 eV, a secondary electron multiplier value of 1541 and at 1.2 scans/s. The cGC system was fitted with a 30 m by 0.25 mm ID fused-silica capillary column coated with DB-1 (0.25 μm) (J & W Scientific, Rancho Cordova, CA, USA). The oven temperature was programmed as follows: (level 1) initial temperature, 80°C; initial hold, 5 min; program rate, 25°C/min; final temperature, 160°C; final hold, 1 min; (level 2) program rate, 4°C/min, final temperature, 275°C; final hold 5 min. Injector and detector temperatures were maintained at 230 and 280°C, respectively.

The other cGC-MS system was a Finnigan MAT Ion Trap Detector (ITD). Spectra were acquired under both (EI) and chemical ionization (CI) conditions. The multiplier voltage was 1400 V, emission current set at 10 μamps and scan rate of 1 s. In the CI mode, methane was used as the reagent gas. The GC was also fitted with a DB-1 capillary column and the oven temperature programmed as described above for the GC-MSD.

Isolation of Minor Alkaloids from South American Coca Leaf

The following was done in duplicate. About 200 g of air-dried coca leaf (≈1.4 g cocaine), powdered to pass a 2-mm sieve, was triturated with 200 mL of an aqueous solution saturated with sodium bicarbonate and then transferred to a 4 L Erhlenmeyer flask. To the flask was added 2 L of water-saturated toluene and then the suspension heated at 50–60°C for several hours with frequent mixing. After cooling, the suspension was filtered through a Buchner funnel and the filtrate set aside. The coca leaf residue was transferred back to the flask and 1 L of water-saturated toluene added. After heating at 50–60°C for 1 h, with frequent mixing, the suspension was filtered as before and the filtrate set aside.

The first toluene extract (~1700 mL) was transferred to the head of a glass chromatographic column (950 mm by 50 mm ID) packed with a homogeneous mixture of 100 mL of 0.36 N sulfuric acid/200 g of Celite 545 and allowed to pass through. The second toluene extract was then passed through the column. About 500 mL of water-saturated toluene followed by an equal volume of water-saturated chloroform were passed through the column. All eluates were discarded.

To the column was added 500 mL of water-saturated chloroform containing 2% diethylamine, followed by 400 mL of water-saturated chloroform; the first 400 mL of eluate were discarded and the final 500 mL collected and saved. After drying the 500-mL eluate over

anhydrous sodium sulfate, it was reduced under vacuum at 50–60°C to a syrupy residue (note: Where applicable, all subsequent column eluates were similarly reduced to residues). The duplicate residues were combined and identified as Fraction CL-A (note: All alkaloid fractions derived from coca leaf are denoted by a CL-prefix).

Fraction CL-A was dissolved in 250 mL of chloroform and extracted with 400 mL of the pH 4.0 acid phthalate buffer. If the pH of the buffer increased, it was adjusted to a pH of 3.9–4.1 with hydrochloric acid and the extraction repeated. The chloroform extract was passed through anhydrous sodium sulfate and set aside. The buffer was extracted with an additional 150 mL of chloroform, which was also dried and combined with the first chloroform extract. The combined extracts were then reduced to a syrupy residue and denoted as Fraction CL-B.

The buffer from above was saturated with sodium bicarbonate and then extracted with 5 by 75-mL aliquots of chloroform, which were combined, dried, and reduced to residue. This residue, Fraction CL-C, was dissolved in 60 mL of chloroform and extracted with 30 mL of pH 4.0 acid phthalate buffer. If the pH of the buffer increased, it was adjusted to a pH of 3.9–4.1 with hydrochloric acid and the extraction repeated. The chloroform phase was set aside and the buffer extracted with an additional 10 mL of chloroform. After combining the chloroform extracts, they were dried, reduced to residue and added to Fraction CL-B above.

To the 30 mL of buffer from above was added 60 g of Celite 545 and then thoroughly mixed and packed into a glass chromatographic column (400 mm L by 40 mm ID) already packed with a homogeneous mixture of 5 mL of pH 4.0 phthalate buffer and 10 g of Celite 545. Water-saturated chloroform was passed through the column until 150–200 mL of eluate was collected; the eluate was dried and reduced to residue, Fraction CL-D.

The column was then basified by elution with a minimal volume of water-saturated chloroform/diethylamine (95/5) followed by 100 mL of water-saturated chloroform. The eluate was dried and reduced to residue, Fraction CL-E (note: This fraction contained highly volatile N-methylpyrrolidine alkaloids, e.g., hygrine, and should not be treated with prolonged periods of above-ambient temperatures).

Fraction CL-D was reconstituted in ca 5 mL of dry chloroform and transferred to the head of a glass chromatographic column (390 by 20 mm ID, with a 250-mL reservoir) packed with: Lower layer—60 g of basic alumina (3.5% water), upper layer—10 g of anhydrous sodium sulfate. Sufficient chloroform was added to the column to collect 150 mL of eluate, Fraction CL-F1; this was followed by the addition of 200 mL of chloroform:acetone (85:15), Fraction CL-F2, followed by 200 mL of chloroform:acetone (1:1), Fraction CL-F3, and finally 200 mL of acetone, Fraction CL-F4. All fractions were reduced to residue.

Fraction CL-B was reconstituted in a minimal volume of water-saturated chloroform and transferred to the head of a glass chromatographic column (600 by 44 mm ID) packed with: Lower layer—mixture of 10 mL of 1 M sodium bicarbonate/20 g Celite, upper layer—mixture of 50 mL of 1 M hydrochloric acid/2 M sodium chloride/100 g Celite. Sufficient water-saturated chloroform was added to the column to collect 100 mL of eluate, which was dried and reduced to residue. The residue was then redissolved in a small volume of water—saturated chloroform and transferred to the head of a second chromatographic column (ca 400 by 22 mm ID) packed with a mixture of 5 mL of 0.36 N sulfuric acid/10 g Celite. About 50 mL of water—saturated chloroform was added to the column and the eluate, which contained colored matter, was discarded. Water—saturated chloroform/diethylamine (99/1) was added to the column, resulting in the movement of a dark-colored band down the column. This band was collected, dried and reduced to residue Fraction CL-G. Scheme 1 outlines the coca leaf fractions.

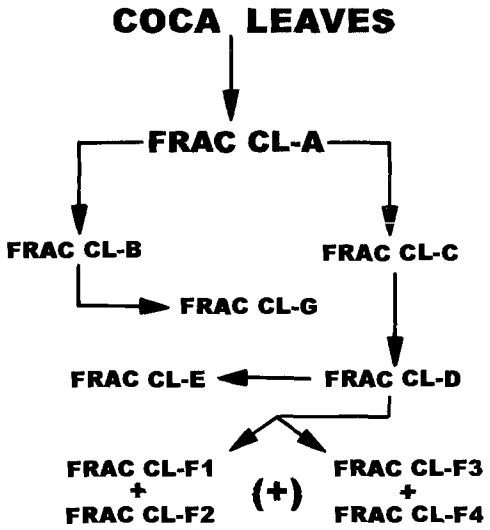
Isolation of Coca Alkaloids from E Harz

About 200 g of *E Harz* (~80 g cocaine) was dissolved in 500 mL of chloroform and extracted with 2000 mL of the pH 4.0 acid phthalate buffer. If the pH of the buffer increased, it was adjusted to a pH of 3.9–4.1 with hydrochloric acid and the extraction repeated. After setting the chloroform aside, the buffer was extracted with 4 by 200-mL aliquots of chloroform and the buffer saved. The combined chloroform extracts were dried and reduced to residue as Fraction EH-1a (note: all alkaloid fractions derived from *E Harz* are denoted by an EH-prefix). This fraction was further subjected to ion-pairing chromatography (similar to that described above for Fraction CL-A) giving Fraction EH-1a'.

The remaining buffer was basified with 200 g of sodium bicarbonate and extracted with 4 by 200-mL aliquots of chloroform. After drying, the combined extracts were reduced to a syrupy residue which was reconstituted in 750 mL of chloroform and extracted with an equal volume of pH 4.0 acid phthalate buffer. If the pH of the buffer increased, it was adjusted to pH 3.9–4.1 with hydrochloric acid and the extraction repeated. The buffer was then back-extracted with an additional 3 by 200-mL aliquots of chloroform, discarding the latter. The buffer was basified with 100 g of sodium bicarbonate and extracted with 3 by 200-mL aliquots of chloroform, which were dried and reduced to residue, Fraction EH-1b.

Fraction EH-1b was reconstituted in a minimal volume of chloroform and transferred to the head of a glass chromatographic column (ca 965 by 50 mm ID) packed with: bottom layer—600 g of basic alumina (4.0% H_2O), upper layer—250 g of anhydrous sodium sulfate. Sufficient chloroform was added to collect 600 mL of eluate—Fraction EH-1A; followed by 600 mL of chloroform:acetone (85:15)—Fraction EH-1B; 600 mL of chloroform:acetone (1:1)—Fraction EH-1C; 600 mL of acetone—Fraction EH-1D; and 600 mL of acetone:methanol (1:1)—Fraction EH-1E. All fractions were reduced to residue.

An appropriate quantity of Fraction EH-1C was dissolved in a



SCHEME 1—*Derivation of alkaloid fractions from South American coca leaf (See Table 2).*

minimal volume of chloroform and transferred to the head of a chromatographic column (ca 457 by 38 mm ID) packed with: Lower layer—200 g of neutral alumina (4% H_2O), upper layer—50 g of anhydrous sodium sulfate. Chloroform was added to collect 200 mL of eluate—Fraction EH-1-C1, followed by 200 mL of chloroform:acetone (85:15)—Fraction EH-1-C2; 200 mL of chloroform:acetone (1:1)—Fraction EH-1-C3; and 200 mL of acetone—Fraction EH-1-C4. All fractions were dried and reduced to residue.

An appropriate quantity of residue from Fractions EH-1A and EH-1B were each dissolved in a minimal volume of hexane:methylene chloride (9:1) by warming on steam bath. Crystallization occurred upon cooling and the crystals removed via centrifugation. The mother liquors were reduced to residues, which were then each subjected twice more to similar recrystallization. The final mother liquors were reduced to residues and identified as Fractions EH-2A and EH-2B, respectively. Scheme 2 outlines the *E Harz* fractions.

Derivatization and cGC-MS Analyses of Ion-Pair, Alumina and Recrystallization Fractions from South American ECVC and E Harz

Each residue from the various alumina and ion-pair column and recrystallization fractions was dissolved in an appropriate volume of chloroform and divided into two aliquots. One aliquot was subjected directly to cGC-MS analysis under previously described conditions. To the other aliquot was added an equal volume of MSTFA, the solution heated at 75°C for 30 min, then subjected to cGC-MS analysis.

Results and Discussion

Sources of Coca

Three sources of coca were used in this study for alkaloid isolation and characterization. Two were freshly-harvested coca leaf, known to be *E. coca* var. *coca*, cultivated in the Upper Huallaga Valley of Peru and the Chapare region of Bolivia. The third source was a commercially-processed, partially-refined extract of South American coca leaf denoted by the manufacturer as *E Harz*. A distinct advantage of *E Harz* in this study was that it was a concentrated extract of multi-kilogram quantities of coca leaf and, therefore, its analysis was much less labor-intensive compared with a cocaine-equivalent quantity of coca leaf. A disadvantage of *E Harz* versus coca leaf, however, was that its commercial preprocessing resulted in the loss of some trace-level coca alkaloids.

Coca Alkaloids

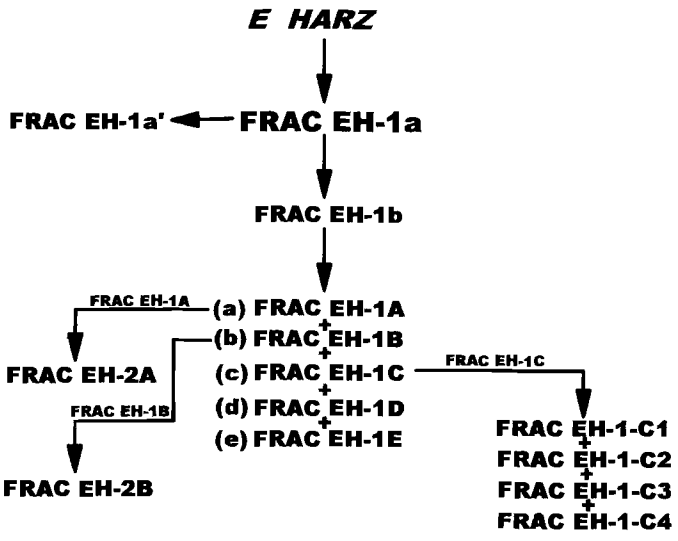
In addition to cocaine, other established alkaloids present in South American ECVC and its extracts include *cis-* and *trans-*cinnamoylcocaine, tropacocaine, the 11 isomeric truxillines, the trimethoxy-substituted tropane alkaloids, ecgonine methyl ester and cuscohygrine, an N-methylpyrrolidine alkaloid. Using the methodology herein, we have detected these, as well as an additional 125–150 new trace-level alkaloids, including the titled alkaloids seen in Fig. 1, namely, nicotinoyl-, 2′-pyrroloyl-, and 2′- and 3′-furanoylecgonine methyl ester. The alkaloidal composition of the various isolated fractions derived from *E Harz* and South American ECVC are found in Tables 1 and 2.

Extraction of Coca Alkaloids from Leaf and Chloroform/Buffer Partitioning

We have previously discussed the advantages of using toluene for the extraction of alkaloids from coca leaf (2). In addition to the high efficiency of alkaloid extraction, the use of toluene resulted in the quantitative retention of virtually all alkaloids on an acid/Celite column, obviating the need for any intervening evaporation steps associated with conventional solvent extractions. This helped to minimize target alkaloid degradation and artifact formation, while concomitantly allowing for the removal of non-alkaloidal and other unwanted plant constituents.

Repetitive partitioning of the isolated leaf alkaloids between chloroform and a pH-4.0 acid phthalate buffer allowed for the removal of significant amounts of unwanted cocaine and the cinnamoylcocaines (which were present in the chloroform phase). Also retained in the chloroform were a host of trace-level alkaloids that exhibited ion-pairing characteristics, i.e., they could be eluted from HCl/NaCl/Celite columns as alkaloid-hydrochloride ion-pairs when using chloroform as eluant. Several of these were recently characterized as 3′,4′,5′-trimethoxybenzoyl- and cinnamoyl-substituted tropane alkaloids (8) (Table 2).

The pH-4.0 buffer phase held the bulk of new trace-level coca alkaloids, including three of the titled compounds. Cocaine and the cinnamoylcocaines were present at reduced levels, as were



SCHEME 2—*Derivation of alkaloid fractions from E Harz (See Table 1).*

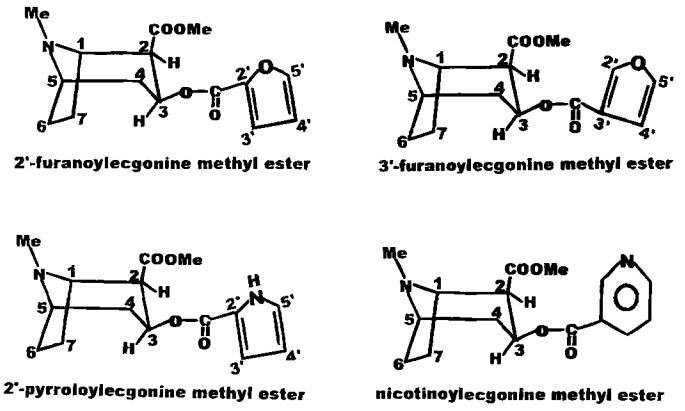


FIG. 1—*Structures for titled alkaloids.*

TABLE 1—*Alkaloidal composition of* E HARZ *fractions.*

Fraction	Alkaloidal Composition
EH-1a	Numerous new trace-level 3-oxo- and 2-carbomethoxy-3-oxo-tropane alkaloids, especially 3′,4′,5′-trimethoxy-aromatic substituted (e.g., 3′,4′,5′-trimethoxycocaine (8)); a useful chemical characteristic of these alkaloids is their quantitative elution from bulk cocaine matrix using chloroform eluant and stationary phase of 1N HCl/2N NaCl/Celite - referred to as "ion-pairing" alkaloids (note: None of these alkaloids possessed derivatizable functional groups); also present were high/interfering levels of cocaine, the cinnamoylcocaines, and tropacocaine.
EH-1a′	Same as Fraction EH-1a, except levels of cocaine and the cinnamoylcocaines were significantly reduced to near-trace levels.
EH-1b	Approx. 125–150 new trace-level carbomethoxy- and 3-oxo-tropane alkaloids, including (a) nicotinoyl-, 2′pyrroloyl-, 2′- and 3′-furanoylecgonine methyl ester (note: these are titled alkaloids; see Fig. 1), (b) approx. 15–20 alkaloids with substitution at C-3 being suspected nonaromatic saturated and unsaturated straight-chain hydrocarbons (note: None possess derivatizable functional groups); (c) approx. 50–75 2-carbomethoxy-3-oxo-tropane alkaloids with the C-3 substituent being hydroxy-substituted non aromatic, 10-carbon dienes and trienes (note: All hydroxy groups readily formed TMS derivatives); (d) alkaloids with TMS-derivatizable hydroxy-substitution on tropane ring (e.g., 1-hydroxytropacocaine; see Reference (13)), (e) 2-carbomethoxy-3-oxo-tropane alkaloid(s) with derivatizable hydroxy substitution on aromatic ring of C-3 substituents; (f) high molecular weight (MW > 600), cyclobutane(?)-type tropane alkaloids with hydroxy substituent(s) (note: These alkaloids did not chromatograph using conventional GC, even with MSTFA derivatization; however, their presence was suspected by the appearance of a significant ecgonidine methyl ester peak in the chromatogram, suggesting the presence of a truxilline-type alkaloid); and (g) N-demethylated tropane alkaloids, dominated by the presence of N-norcocaine. Also present were significant and interfering levels of cocaine and cinnamoylcocaines (however, levels much lower than in Fraction 1a). Also present were relatively high quantities of the isomeric truxillines.
EH-1A	Trace levels of 2′- and 3′-furanoylecgonine methyl ester (see Fraction EH-1b); approx. 15–20 alkaloids with substitution at C-3 being suspected nonaromatic saturated and unsaturated straight-chain hydrocarbons (note: See Fraction EH-1a); significant levels of cocaine and cinnamoylcocaines; only small quantities of the truxillines; tropacocaine present; none of the trace-level alkaloids possessed derivatizable functional groups.
EH-1B	Similar to Fraction EH-1A, except presence of 2′- and 3′- furanoylecgonine methyl ester appeared enhanced; somewhat decreased amounts of cocaine/cinnamoylcocaines; truxillines levels enhanced.
EH-1C	Trace levels of nicotinoylecgonine methyl ester and 2′-pyrroloylecgonine methyl ester (see Fraction EH-1b); 2′-pyrroloylecgonine methyl ester forms N-TMS derivative with MSTFA (note: Underivatized 2′-pyrroloylecgonine virtually co-elutes with cocaine; as N-TMS derivative peak is shifted to transparent retention window-see Fig. 3 and Table 3); also present were further reduced levels of cocaine/cinnamoylcocaines; substantial amounts of truxillines (note: The truxillines undergo thermal degradation in GC injection port, producing early-eluting ecgonidine methyl ester and a multiplicity of artifact peaks eluting between cocaine and the cinnamoylcocaines); hydroxy-substituted alkaloids begin to appear in this fraction.
EH-1D	This fraction included virtually all of the hydroxy-substituted carbomethoxy tropane alkaloids, 75–100 in number (note: All appeared to form O-TMS derivatives); these included all the alkaloids cited above in Fraction EH-1b:(c)-(f); only trace-levels of cocaine, cinnamoylcocaines, and the truxillines present.
EH-1E	Present in this fraction were only insignificant levels of hydroxy-substituted tropane alkaloids; high molecular weight tropane alkaloids may be present; fraction dominated by presence of N-norcocaine.
EH-1-C1	Present were mostly cocaine and the cinnamoylcocaines.
EH-1-C2	One of the titled alkaloids, nicotinoylecgonine methyl ester, appeared for the first time along with significant amounts of cocaine, cinnamoylcocaines, the truxillines and tropacocaine.
EH-1-C3	Two of the titled alkaloids, nicotinoylecgonine methyl ester and 2′-pyrroloylecgonine methyl ester, were found in this fraction (note: See Fig. 3 for chromatography), along with the truxillines and only trace levels of cocaine, cinnamoylcocaines, and tropacocaine.
EH-1-C4	Mostly insignificant levels of the hydroxy-substituted tropane alkaloids.
EH-2A/EH-2B	Recrystallization of combined Fractions EH-1A and 1-B and isolation of mother liquor resulted in Fraction EH-2A/EH-2B; this fraction held enhanced levels of 3′-furanoylecgonine methyl ester and diminished amounts of 2′-furanoylecgonine methyl ester; also found were enhanced quantities of about 15–20 carbomethoxytropane alkaloids with substitution at C-3 being suspected nonaromatic saturated and unsaturated straight-chain and branched hydrocarbons (see Fig. 2 (b)).

significant amounts of the truxillines, the non-tropane alkaloids cuscohygrine and hygrine and various new N-methylpyrrolidine compounds. The greatest number of new alkaloids in the buffer were believed to be hydroxy-substituted 3-carboxy- and 2-carbomethoxy-3-carboxy-tropane alkaloids (Table 2). This hydroxy substitution was found primarily on various substituents at the C-3 site; and also at different sites on the tropane ring.

Chloroform/Buffer Partitioning of E Harz

Because *E Harz* was already a coca leaf concentrate, toluene was not needed in its workup. Instead, its analysis began with the direct partitioning of the matrix between chloroform and the pH-4.0 buffer. The alkaloidal content of both the chloroform and buffer phases of *E Harz* were similar in many respects to that for coca leaf, except for the near-absence of the N-methylpyrrolidine alkaloids in *E Harz* (Table 1). Furthermore, because *E Harz* represented many more cocaine equivalents compared to the coca leaf analyzed in this study, a greater number of trace-level tropane alkaloids were detected in the former matrix.

Refinement of Partitioned Alkaloids from E Harz *and Coca Leaf*

The initial chloroform/pH-4.0 buffer partitioning of the isolated total alkaloids from the coca leaf and *E Harz* was followed by further refinement using: (a) Ion-pairing column chromatography with a stationary phase of 1 M HCl/2 M NaCl/Celite 545, and/or (b) basic and neutral alumina column chromatography, and (c) recrystallization of selected alumina fractions. In the latter part of this study, silica adsorption and buffer partitioning column chromatography, along with preparative HPLC, were used for alkaloid refinement.

TABLE 2—*Alkaloidal composition of South American coca leaf fractions.*

Fraction	Alkaloidal Composition
CL-A	This chloroform fraction held the total of coca leaf alkaloids, both major and new trace-level ones, including 2′- and 3′-furanoyl- and nicotinoylecgonine methyl ester (note: Insufficient sample precluded the detection of 2′-pyrroloylecgonine methyl ester).
CL-B	After pH-4 buffer extraction of Fraction CL-A, the remaining chloroform phase (Fraction CL-B) held "ion-pairing" alkaloids (as described for *E Harz* Fractions EH1a and EH-1a′ in TABLE 1) and high levels of cocaine, cinnamoylcocaines and tropacocaine.
CL-C	Extraction of Fraction CL-A with pH-4 buffer transferred most of the trace-level alkaloids (especially hydroxy-substituted ones) to the buffer, Fraction CL-C; these included three of the titled alkaloids, hydroxy-substituted 3-oxo- and 2-carbomethoxy-3-oxo-tropane compounds and N-methylpyrrolidine alkaloids; also present were significant levels of cocaine, cinnamoylcocaines and the truxillines.
CL-D	Found in this fraction were all the alkaloids cited in Fraction CL-C, excepting the N-methylpyrrolidine alkaloids, e.g., cuscohygrine, and some truxillines.
CL-E	This fraction consisted primarily of the N-methylpyrrolidine alkaloids, such as cuscohygrine, hygrine and related unidentified compounds (note: all these alkaloids were characterized by the presence of an MS base peak at m/z 84; representing the N-methylpyrrolidinium ion; the remaining spectra exhibited only very weak molecule and fragment ions).
CL-F	Found were hydroxy-substituted 3-oxo- and 2-carbomethoxy-3-oxo-tropane alkaloids
CL-G	Contained "ion-pair" alkaloids, e.g., 3′,4′,5′-Trimethoxycocaine.

TABLE 3—*Gas chromatographic retention data for titled alkaloids and other coca constituents.*

Alkaloid/Compound	Retention Time (Min)
Ecgonidine methyl ester	14.64
Ecgonine methyl ester	15.50
Ecgonine methyl ester—TMS	16.55
Tropacocaine	19.51
3′-Furanoylecgonine methyl ester	19.72
2′-Furanoylecgonine methyl ester	20.18
2′-Pyrroloylecgonine methyl ester	21.03
Cocaine	21.06
Nicotinoylecgonine methyl ester	21.36
N-TMS-2′-Pyrroloylecgonine methyl ester	21.50
Truxilline degradation artifacts	21.50
	21.89
	22.06
cis-Cinnamoylcocaine	22.14
Truxilline degradation artifacts	22.27
	22.30
	22.51
trans-Cinnamoylcocaine	23.06

*Chromatographed on 30 m by 0.25 mm/GC-MSD; see Experimental for GC conditions.

Most of the analytical effort was focused upon *E Harz*, as opposed to the leaf, as it was the source of many more trace-level alkaloids. Thus, greater numbers of new alkaloids were characterized in that matrix compared to the leaf. However, the quantitative ratio of trace-level-alkaloids-to-cocaine was significantly lower in *E Harz*, requiring a more extensive alumina column "cleanup" compared to the leaf.

Chemical Derivatization

Trimethylsilylation was necessary for the detection and facile characterization of certain trace-level tropane alkaloids, notably those which were hydroxy-substituted, e.g., the hydroxycocaines (7). The derivatization of virtually all of these compounds using MSTFA was rapid and went to completion. The resultant TMS derivatives exhibited marked improvement in GC behavior, enhancing their mass spectral characterization. It was interesting to note that cuscohygrine formed a high-yield TMS derivative after prolonged heating in MSTFA. This compound was presumed to be the O-TMS derivative of the enolate of cuscohygrine. An improvement in chromatography for this derivative was also observed. We have previously used heptafluorobutyrylation in the detection of the hydroxycocaines (7) and, recently, the novel 1-hydroxytropacocaine (14); not surprisingly, these alkaloids were detected in both *E Harz* and coca leaf.

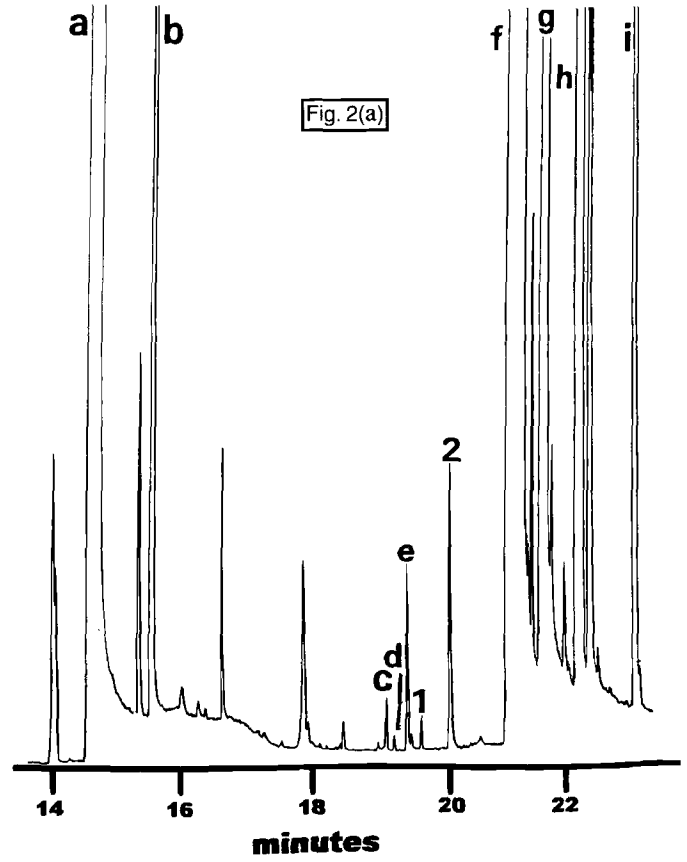
Mass Spectral Analysis

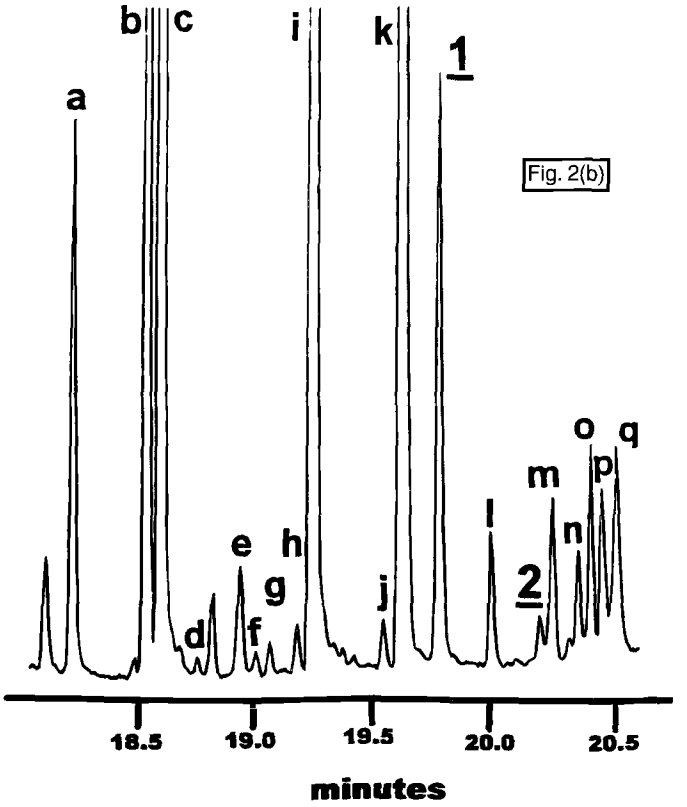
After chemical derivatization, all alumina and ion-pair column and recrystallization fractions were subjected to El and Cl mass spectrometry. A review of the mass spectra allowed for their classification as described below. The most diagnostic tropane alkaloid fragment ions were at 82 and 182 Daltons, representing a 2-carbomethoxy-tropane moiety. A base peak ion at 94 Daltons suggested substitution (other than at C-2 and C-3) on the tropane moiety. A base peak ion at 84 Daltons inferred the presence of an N-methylpyrrolidine moiety. The titled alkaloids were identified via comparison of their mass spectra with those of the appropriate synthesized standards. The alkaloidal composition of the various fractions from *E Harz* and coca leaf are described below.

New Alkaloids in E Harz

Table 1 lists the alkaloidal composition for the *E Harz* fractions (See also Scheme 2). It is apparent that *Fractions EH-1a* and *EH-1b* represent crude fractions of coca alkaloids, including about 125–150 new tropane alkaloids, most of which were found in *EH-1b*. With each successive column fraction, the trace-level alkaloids were further refined. Thus, *Fractions EH-1A*, *EH-1B* and *EH-2A/EH-2B* held both 2′- and 3′-furanoylecgonine methyl ester along with some unknown trace-level and known major alkaloids. All compounds in these fractions exhibited fragment ions at m/z 82, 182 and 198, diagnostic for 2-carbomethoxy-3-oxo-tropane moieties.

Figure 2(a) illustrates the chromatography for the two furanoyl alkaloids (peaks 1 and 2), along with other constituents, from *E Harz*. It should be noted that in both *E Harz* and ECVC, 2′-furanoylecgonine methyl ester was far more abundant than its 3′-isomer. Only after recrystallization of *Fractions EH-1A* and *EH-1B*





to yield *EH-2A* and *EH-2B* (Table 1) was the 3′-isomer enhanced relative to the 2′ compound. This is illustrated by peaks *1* and *2* in Fig. 2(b). About 15–20 other new trace-level alkaloids (peaks a–q in Fig. 2(b)), all bearing a 2-carbomethoxy-3-oxo-tropane moiety, were also enhanced after recrystallization; these will be addressed in a future report.

The two other titled compounds in *E Harz*, nicotinoyl- and 2′-pyrroloylecgonine methyl ester (Fig. 1), were detected in *Fractions EH-1C*, *EH-1-C2*, and *EH-1-C3*. Their chromatography (from *Fraction EH-1-C3* in Table 1), along with other known alkaloids, is illustrated in Fig(s). 3(a) and (b). Of the four titled alkaloids, only the pyrrole compound formed a trimethylsilyl (TMS) derivative, i.e., N-TMS. This was fortuitous in that the underivatized 2′-pyrroloylecgonine methyl ester practically coeluted with cocaine (Fig. 3(a)); however, the N-TMS derivative was shifted to a transparent retention window (Fig. 3(b)). Some difficulty was noted in the MSTFA derivatization of the pyrroyl-alkaloid, perhaps due to matrix effects. This is demonstrated in Fig. 3(a) which shows only a low yield of the N-TMS derivative (peaks 1 and 3), whereas in Fig. 3(b), which was a more highly-refined extract, a high yield conversion was noted (peaks 1 and 3).

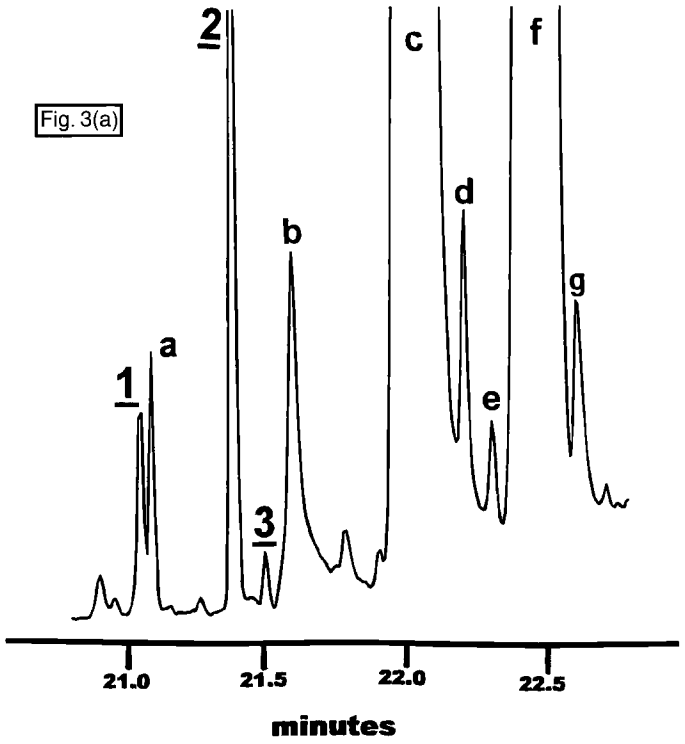
Dominating the chromatogram illustrated in Fig. 3(a) are artifact peaks (b–g) which arose from the thermal degradation of the isomeric truxillines in the GC injection port. Because of their thermal lability, the truxillines are difficult to chromatograph intact. Repetitive alumina column chromatography of the fraction represented in Fig. 3(a) resulted in a significant diminution of these artifacts as evidenced in Fig. 3(b).

Also seen in Table 1, Fraction EH-1a′ is a group of about 25–30 alkaloids which are able to form hydrochloride ion-pairs on hydrochloric acid/sodium chloride/celite columns that can be quantitatively eluted with chloroform. Most of these are new coca alkaloids possessing a 3-oxo- or 2-carbomethoxy-3-oxo-tropane moiety but with no derivatizable substituents. Several of these were recently identified as 3′, 4′, 5′-trimethoxy-substituted analogues of cocaine, tropacocaine and *cis-/trans*-cinnamoylcocaine (8). The characterization of some of the other new alkaloids in this fraction will be the subject of a future report.

The largest group of new trace-level alkaloids found in *E Harz*, about 75–100 in number, were hydroxy-substituted 3-oxo- and 2-carbomethoxy-3-oxo-tropane compounds. They were characterized by the presence of a TMS fragment ion at m/z 73 and major ions at m/z 82/182 or 124. In addition, most exhibited definitive molecule ions. These alkaloids were first isolated as a crude mixture with cocaine, cinnamoylcocaines, and the truxillines in Fraction EH-1b (Table 1). Further alumina column refinement placed

FIG. 2(a)—*cGC-MSD chromatogram of* E Harz *Fraction EH-1B illustrating 2′- and 3′-furanoylecgonine methyl ester and other coca alkaloids.*[a] [a]*Peak identification: 1 = 3′-furanoylecgonine methyl ester, 2 = 2′-furanoylecgonine methyl ester, a = ecgonidine methyl ester (artifact), b = ecgonine methyl ester, c & d = unidentified 2-carbomethoxy-3-oxo-tropane alkaloids, e = tropacocaine, f = cocaine, g = truxilline thermal degradation product and h & i =* cis- *and* trans-*cinnamoylcocaine. See EXPERIMENTAL for cGC-MSD conditions. See Table 3 for retention times and Table 1 for fraction identification.*

(b) *cGC-MSD chromatogram of* E Harz Fractions EH-2A/EH-2B *illustrating enhanced 3′-furanoylecgonine methyl ester and suppressed 2′-furanoylecgonine methyl ester as well as unknown tropane alkaloids.*[a] [a]*Peak identification: 1 = 3′-furanoylecgonine methyl ester, 2 = 2′-furanoylecgonine methyl ester and a–q = unknown 2-carbomethoxy-3-oxo-tropane alkaloids. See EXPERIMENTAL for cGC-MSD conditions. See Table 3 for retention times and Table 1 for fraction identification.*



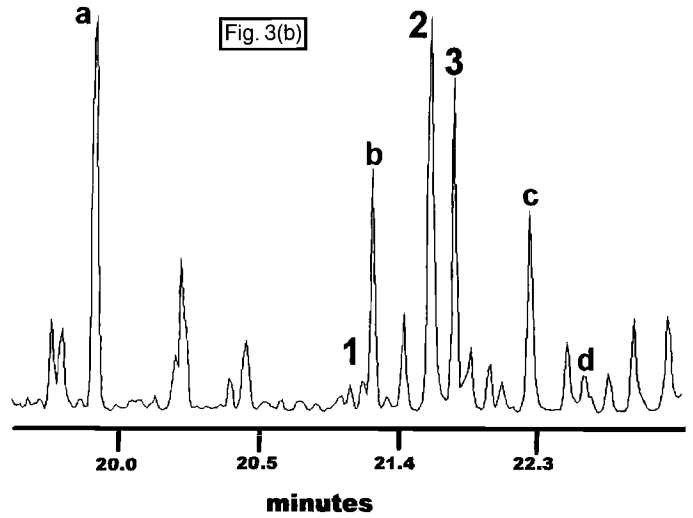


FIG. 3(a)—*cGC-MSD chromatogram of* E Harz Fraction EH-1-C3 *illustrating 2'-pyrroloylecgonine methyl ester and nicotinoylecgonine methyl ester along with truxilline thermal degradation products.*[a]

[a]*Peak identification: 1 = 2'-pyrroloylecgonine methyl ester, 2 = nicotinoylecgonine methyl ester, 3 = N-TMS derivative of 2'-pyrroloylecgonine methyl ester (note low yield of derivative), a = cocaine and b–f = truxilline thermal degradation products. See Table 3 for retention times and Table 1 for fraction identification.*

FIG. 3(b)—*GC-ITD chromatogram of* E Harz *refined* Fraction EH-1-C3 *illustrating nicotinoylecgonine methyl ester and the enhanced N-TMS derivative of 2'-pyrroloylecgonine methyl ester.*[a]

[a]*Peak identification: 1 = 2'-pyrroloylecgonine methyl ester, 2 = nicotinoylecgonine methyl ester, 3 = N-TMS derivative of 2'-pyrroloylecgonine methyl ester, a = tropacocaine, b = cocaine, c & d = truxilline thermal degradation products. See Table 3 for retention times and Table 1 for fraction identification.*

these alkaloids in Fraction EH-1D, with only trace levels of the major alkaloids present. For most of these new alkaloids, the hydroxy group was located on the C-3 substituent, believed to be mostly 10-carbon dienes and trienes; however, at least one alkaloid was believed to be hydroxy-substituted on the aromatic ring. In addition, hydroxy substitution on the tropane moiety was seen for five or six alkaloids. These were characterized, in part, by exhibiting a base peak at m/z 94, the absence of ions at m/z 82/182, and weak molecule ions.

Finally, detected in Fraction EH-1D and in Fraction EH-1E were believed to be as many as 25–35 new isomeric, hydroxy-substituted, high-molecular weight tropane alkaloids, probably possessing a cyclobutane ring, e.g., as in the truxillines. Because of their large mass and thermal lability, these compounds were difficult to chromatograph by GC; however, their presence was indicated by the appearance of an ecgonidine methyl ester GC peak, an artifact resulting from the thermal degradation of these alkaloids in the GC injection port. Because of the hydroxy substituent, these compounds could be resolved from the truxillines by alumina column chromatography.

New Alkaloids in South American ECVC

Table 2 lists the alkaloidal composition of the fractions derived from the South American ECVC samples (See also Scheme 1). Because of the limited amount of leaf available, sharply reduced levels of total alkaloids (compared to *E Harz*) were realized. Nonetheless, the trace-level alkaloids that were found in the leaf fractions were also seen in *E Harz*. Furthermore, because the trace-alkaloid-to-cocaine ratio was higher for the leaf, a less rigorous alumina column refinement was necessary compared with *E Harz*. This caused a shift in the alkaloid content of the various leaf column fractions (compared to *E Harz*) in which the titled alkaloids and other trace-level compounds were found.

As seen in Table 2, three of the four titled alkaloids were present in Fraction CL-F2 (because of insufficient sample, detection of 2'-pyrroloylecgonine methyl ester was not possible). Figure 4 illustrates the chromatography for a leaf sample cultivated in Peru's Upper Huallaga Valley. As in *E Harz*, the 2'-furanoyl alkaloid (peak 2) in the leaf sample was at least 5× more abundant than its 3'-isomer (peak 1). Nicotinoylecgonine methyl ester was identified as peak 3. It is interesting to note that, unlike Peru, the furanoyl alkaloids were not detected in the single sample of Chapare Valley (Bolivia) coca examined. Caution should be exercised in rationalizing these results, however, pending the analyses of many more coca samples from Peru, Bolivia, and other coca source countries.

Fraction CL-E (Table 2) consisted of a mixture of the N-methylpyrrolidine alkaloids, with cuscohygrine being by far the most abundant. Hygrine and about a dozen new trace-level N-methylpyrrolidine compounds were also present; some of these also formed TMS derivatives. The mass spectra for these alkaloids were characterized by a base peak at 84 Daltons representing the N-methylpyrrolidinium ion, with the remaining fragment ions being extremely weak. For the most part, detection of molecule ions was problematic, even under CI conditions.

Fraction CL-F3 (Table 2) contained the derivatizable hydroxy-substituted 3-oxo- and 2-carbomethoxy-3-oxo-tropane alkaloids. Many of these alkaloids were detected in Fraction EH-1D of *E Harz* (Table 1). Low levels of cocaine/cinnamoylcocaines and the truxillines were also observed. Although the GC-MS analyses of the Peruvian and Bolivian leaf fractions (Fraction CL-F3) revealed many alkaloids in common, the latter held significantly higher levels of

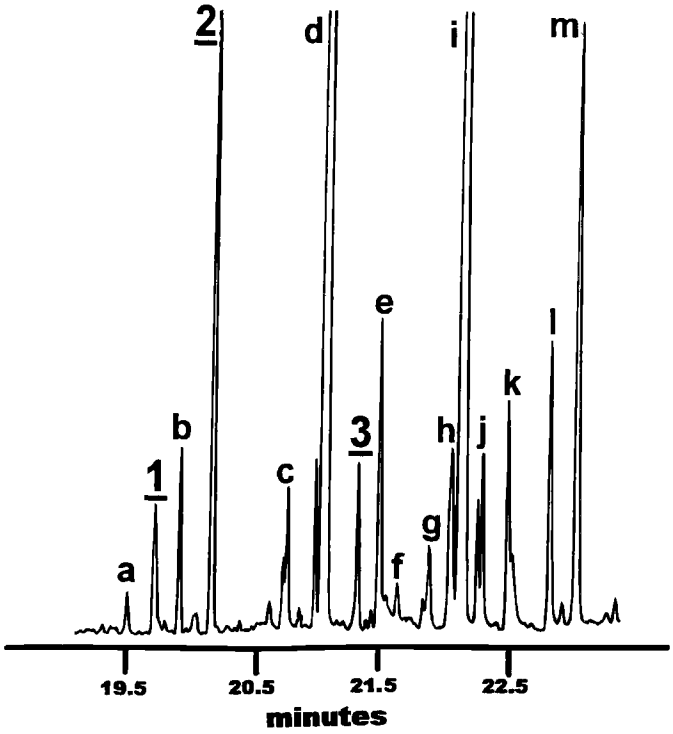


FIG. 4—*cGC-MSD chromatogram of Peruvian coca* Fraction CL-F2 *illustrating 2'- and 3'-furanoylecgonine methyl ester and nicotinoylecgonine methyl ester along with known coca alkaloids.*[a]

[a]*Peak identification: 1 = 3'-furanoylecgonine methyl ester + unidentified N-methylpyrrolidine alkaloid, 2 = 2'-furanoylecgonine methyl ester, 3 = nicotinoylecgonine methyl ester, a = tropacocaine, b = artifact?, c = TMS derivative of unidentified hydroxy-substituted 3-oxo-tropane alkaloid, d = cocaine, e–h, j & k = truxilline thermal degradation product, i =* cis*-cinnamoylcocaine, l = TMS derivative of unidentified hydroxy-substituted 2-carbomethoxy-3-oxo-tropane alkaloid and m =* trans*-cinnamoylcocaine.*

the hydroxy-substituted alkaloids. However, the same caveat applies here as stated above when interpretating these results.

Fraction CL-G held the "ion-pairing" alkaloids from the two leaf samples, including the trimethoxy-substituted 3-oxo- and 2-carbomethoxy-3-oxo-tropane alkaloids. In contrast to the hydroxy-substituted alkaloids, it appeared that the "ion-pairing" alkaloids were more abundant in the Peruvian versus the Bolivian sample.

Structural Characterization of Titled Alkaloids

The four titled alkaloids were subjected to El and Cl mass spectrometry. The El mass spectra are illustrated in Fig(s). 5(a)–(d). All mass spectra were acquired from the most refined fraction(s) containing the titled alkaloids (Tables 1 and 2). All four alkaloids displayed El molecule ions of moderate intensity (confirmed by Cl) and significant ions at m/z 82/182/198, diagnostic for the presence of a 2-carbomethoxy-3-oxo-tropane moiety (Fig. 5). This inferred that the singular difference between these alkaloids and cocaine was at the C-3 substituent. The mass spectra of the four titled alkaloids were also found to be virtually identical with the spectra of their corresponding synthetic standards. The GC retention times of the sample alkaloids were also virtually identical with those of their respective standards. The GC retention times of the titled alkaloids, along with other compounds of interest, are given in Table 3. The 2α- epimers of the titled alkaloids were also prepared and were excluded as possible structures based upon their mass spectra and GC retention times.

The fractions containing the titled compounds and an overview of their mass spectra are summarized below.

2'-Furanoylecgonine Methyl Ester—This alkaloid was found in Fraction EH-1B (Table 1; Fig. 2) and Fraction CL-F2 (Table 2; Fig. 4). As seen in Fig. 5(a), this alkaloid displayed a moderately intense molecule ion at 293 Daltons. A fragment ion at m/z 264 was probably due to the loss of formaldehyde from the molecule ion (15). Lower intensity ions at m/z 262 and 234 were attributed to losses of methoxy and carbomethoxy moieties at C-2. Support for a furan group in the molecule was found at m/z 67 and 95, believed to be furaninium and furanoyl ions, respectively. The spectrum also displayed the intense diagnostic ions for tropanes at m/z 82 and 182 and one of low intensity at m/z 198.

3'-Furanoylecgonine Methyl Ester—This alkaloid, a positional isomer of 2'-furoylecgonine methyl ester, was detected in the same *E Harz* and coca leaf fractions as the 2' isomer (Tables 1 and 2; Fig(s). 2 and 4). Not unexpectedly, its mass spectrum was virtually identical to the 2'-isomer, except for the absence of the m/z 264 ion (loss of formaldehyde from the molecule ion).

Nicotinoylecgonine Methyl Ester—This alkaloid was detected in Fraction EH-1-C3 (Table 1; Fig. 3) and Fraction CL-F2 (Table 2; Fig. 4). Its mass spectrum (Fig. 5(c)) was markedly similar to cocaine. The molecule ion at 304 Daltons (cocaine = 303 Daltons) indicated the probable presence of two nitrogen atoms. This suggested the substitution of a pyridine ring for the benzene ring in cocaine. The presence of the familiar tropane ions at m/z 82/182/198 supported a new substituent at C-3. Further support for a pyridine moiety was found at m/z 78, due to the pyridinium ion (compared to m/z 77 for the phenyl ion in cocaine) and m/z 106, the pyridinoyl ion (compared to m/z 105 for the benzoyl ion in cocaine).

The mass spectra/GC retention times for iso-nicotinoyl- and picolinoylcgonine methyl ester, i.e., the positional isomers of nicotine, excluded these compounds as possible structures.

2'-Pyrroloylecgonine Methyl Ester—This trace-level alkaloid was present in both a crude and refined Fraction EH-1-C3 (Table 1; Fig(s). 3(a), (b)—underivatized and as N-TMS derivative). The presence of the tropane ions at m/z 82/182/198, seen in Fig. 5(d), again suggested a new substituent at C-3. A molecule ion at 292 Daltons inferred the presence of two nitrogen atoms in the molecule. Given this, and the identities of the other three titled alkaloids, it was not unreasonable to suspect the presence of a pyrrole moiety. This was supported by the presence of fragment ions at m/z 66 and 94, representing pyrroyl and pyrroloyl moieties, respectively (compared with m/z 67 and 95 for 2'-furanoylecgonine methyl ester, representing the furaninium and furanoyl ions, respectively). The mass spectrum/GC retention time of 3'-pyrroloylecgonine methyl ester excluded this compound as a possible structure.

Hydrolysis, Derivatization, and Mass Spectral Analyses of Titled Alkaloids

After acquisition of the mass spectral data above, the isolated alkaloids 2'- and 3'-furanoylecgonine- and nicotinoylecgonine methyl ester were subjected to acid hydrolysis. The resultant 2'- and 3'-furanoic and nicotinic acids were isolated and subjected to trimethylsilylation. The mass spectra and GC retention times of their O-TMS derivatives were virtually identical to the TMS derivatives of their respective standards, giving confirmation of the titled alkaloids.

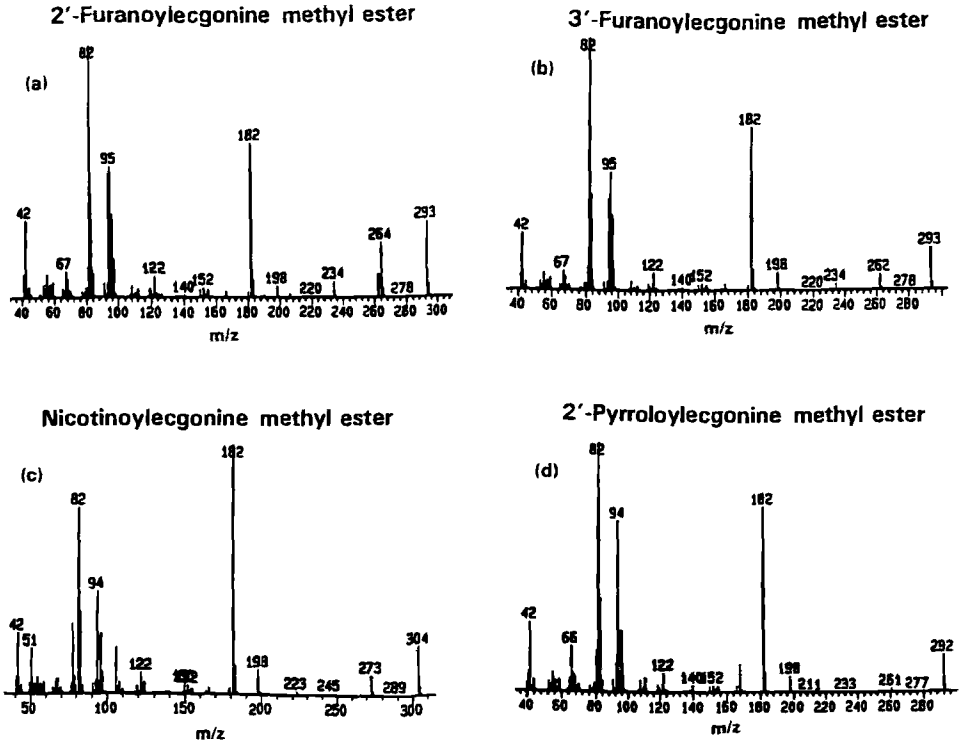


FIG. 5—*cGC-MSD electron ionization mass spectra of titled alkaloids.*

Summary

Four new trace-level alkaloids of South American *Erythroxylum coca* var. *coca*, namely, 2′- and 3′-furanoylecgonine methyl ester, nicotinoyl- and 2′-pyrroloylecgonine methyl ester, have been partially isolated and structurally characterized by electron and chemical ionization mass spectrometry and by comparison of their mass spectra and GC retention times with synthesized standards. Detailed methodology used to detect an additional 125–150 new trace-level 3-oxo- and 2-carbomethoxy-3-oxo-tropane alkaloids in coca was also presented.

Acknowledgments

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Additional information and reprint requests:
James M. Moore
Special Testing and Research Laboratory
7704 Old Springhouse Rd.
McLean, VA 22102-3494