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Review

In-depth chromatographic analyses of illicit cocaine and its precursor, coca leaves

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Abstract

Chromatographic methodology used for the in-depth alkaloid analyses of coca leaves and for the characterization of alkaloidal impurities and manufacturing by-products in illicit refined cocaine samples is reviewed. This includes liquid–liquid partition and liquid–solid adsorption column chromatography, packed- and capillary-column gas chromatography with flame-ionization, electron-capture, nitrogen–phosphorous and mass spectrometric detection, and high-performance liquid chromatography with ultraviolet detection. The rationale supporting the presence and determination of processing impurities/by-products in cocaine samples is discussed, and chromatographic methodology used for the development of drug impurity signature profiles is presented.

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1. Introduction

Chromatography has played a dominant role in forensic drug chemistry over the past 25–30 years. This not only pertains to routine drug analyses but, more recently, to the comprehensive characterization of trace-level manufacturing impurities in illicit drugs. This review focuses upon chromatographic methodology reported for the detection, characterization and determination of manufacturing impurities in one of those drugs, illicit refined cocaine. Also presented are procedures that have been reported recently for the development of cocaine impurity signature profiles. Since illicit cocaine is derived primarily from the South American coca leaf, methods describing the analysis of both known and recently identified alkaloids in that matrix are also discussed. Concomitantly, the relationship between cocaine manufacturing impurities and alkaloids of the coca leaf is presented. For comparison purposes, the analyses of cocaine-bearing cultivars grown in a greenhouse environment is also reviewed.

As a preface to this review, the rationale behind the characterization of drug manufacturing impurities will be presented, along with a brief discussion of how these impurities are generated. Also included will be an overview of both classical and state-of-the-art chromatographic techniques utilized in forensic drug chemistry. The preface will conclude with a cursory review of both chemical derivatization and artifact formation and the impact they have

upon alkaloid analyses and in the development of drug impurity signature profiles.

2. Background

One of the major goals in forensic drug chemistry/research is the full characterization of manufacturing impurities and by-products in illicit drugs. This includes the structural elucidation of these compounds, their quantitative determination, a rationalization for their presence in drugs, investigation of their chemical stability and an evaluation of chemicals/solvents used in the process. If the drug has a natural product origin, such as cocaine, then the in-depth analysis of the plant may also be warranted. The drugs most frequently studied with regard to manufacturing impurities/by-products have been cocaine [1–38], heroin and amphetamine-type compounds.

A thorough analysis of drug processing impurities can provide both tactical and strategic intelligence data to drug enforcement personnel. These include: (1) the ability to compare drug seizures from similar or disparate areas to determine if they were derived from a common source; such information is useful in the development of drug conspiracy cases for eventual criminal prosecution; (2) data derived from processing impurities may allow for the determination of the drug's geographic origin; this applies especially to drugs that have natural product origins, such as cocaine and heroin; (3) tracing of drug distribution routes; (4) an evaluation of the drug's precursor and/or intermediate chemicals used in its manufacture/synthesis; this allows for the monitoring of such chemicals to determine if their use is for legitimate or illicit purposes; (5) the possibility, in some cases, to differentiate between a drug that was manufactured illicitly and that same drug diverted from legitimate pharmaceutical sources; and (6) some illicit drug manufacturing impurities may have serious health implications, even more so than the parent drug; the characterization of such compounds could assist in understanding their toxicology, as a prelude to medical treatment.

3. Origin of manufacturing by-products and impurities in illicit refined cocaine

During the clandestine manufacture of illicit refined cocaine from coca leaves, manufacturing by-products are created that can be detected in the final product. These by-products can be formed by a variety of chemical processes. One of the most common is hydrolysis of the parent drug, which can occur during the manufacture/synthesis or while in storage. Hydrolysis usually occurs at ester linkages; thus, hydrolysis of cocaine yields benzoylecgonine, ecgonine methyl ester, ecgonine and benzoic acid [2,3,38]. Minor alkaloids co-extracted from the coca leaf along with cocaine can also undergo hydrolysis at ester sites. These alkaloids include the cinnamoylcocaines, tropacocaine and the truxillines, among others. Other impurities are the result of oxidative processes. For example, the action of strong oxidants such as peroxides, present as impurities in solvents such as diethyl ether, may cause some tertiary N-methyl amine drugs to undergo N-demethylation. This creates a class of impurities sometimes referred to as an "N-nor" series [39]. The use of potassium permanganate during the processing of illicit cocaine can result in the oxidation of cocaine to yield trace levels of N-formylnorcocaine [24,28]. Other manufacturing by-products are the direct result of epimerization reactions. Thus, ecgonine and ecgonine methyl ester may undergo epimerization at their C-2 positions in the presence of strong base to form their pseudo-epimers [22,40]. Finally, rearrangement reactions may occur during the manufacturing process. Thus, the presence of N-benzoylnorecgonine methyl ester in cocaine samples is believed to be the result of the base-assisted benzoyl migration from the C-3 position to the nitrogen site of N-norcocaine [28]. The levels of different manufacturing by-products in refined cocaine samples vary significantly, about 2–3

orders of magnitude, with the hydrolytes of cocaine being the most abundant.

In addition to manufacturing by-products, refined drugs that have natural product origins are usually contaminated with alkaloidal impurities. When cocaine is extracted from the South American coca leaf, minor alkaloids that are also present in the leaf "survive" the manufacturing process and can be detected in refined samples. These include the cinnamoylcocaines, truxillines, tropacocaine, hydroxycocaines and trimethoxy-substituted alkaloids [1,14,33,36,38]. These alkaloidal impurities vary widely in concentration in illicit refined cocaine samples, from as high as 5–10% to below 10^{-3}% (relative to cocaine).

Yet another source of illicit cocaine contamination is due to processing solvents. The solvent residues that are present at trace levels in illicit refined cocaine samples are believed to appear during the conversion of cocaine base to cocaine hydrochloride. Using nuclear magnetic resonance spectroscopic techniques and headspace gas chromatography–mass spectrometry, solvents that have been detected and quantified include acetone, methyl ethyl ketone, toluene and diethyl ether, among others [41–44].

4. Chromatographic analysis of drug manufacturing impurities and by-products

Chromatography is an integral part of methodology developed for the in-depth analyses of manufacturing impurities and by-products in illicit drugs. This encompasses classical techniques, including various types of column chromatography, and modern applications using capillary gas chromatography (cGC), high-performance liquid chromatography (HPLC) and, most recently, capillary electrophoresis (CE).

4.1. Column chromatography

Classical chromatographic techniques, such as column chromatography, have been utilized over the years for the analyses of many and varied complex matrices. Two types of column chromatography have been most relevant in forensic drug research, namely, liquid–liquid partition and liquid–solid adsorption chromatography. The former type is usually a combination of ion-pairing, "trap" and liquid–liquid partition column chromatography. Typically, cylindrical glass columns (of widely varying lengths and diameters) are packed with a mixture of an acidic or basic aqueous phase and a stationary support of Celite 545, an inert diatomaceous earth. In ion-pairing chromatography the stationary phase is generally a mixture of dilute hydrochloric or nitric acid and Celite 545, while the mobile phase is halogenated and includes chloroform or methylene chloride. Typically, in ion-pairing chromatography an amine drug or basic impurity is eluted quantitatively from the chromatographic column as a hydrochloride or nitrate ion pair. In "trap" chromatography the drug or by-product is quantitatively retained, or "trapped", on the column. For this to occur the compound must be basic or acidic in character. Typical column packings for trap chromatography include mixtures of Celite 545 with dilute inorganic or organic acids or bases, *e.g.*, sulfuric, hydrochloric, nitric, citric and tartaric acids; bases include sodium hydrogencarbonate, sodium carbonate and sodium hydroxide. Neutral compounds, depending upon their polarity and/or solubility characteristics, can also undergo liquid–liquid partition column chromatography. In practical terms, most parent drug/manufacturing impurity separations using Celite column chromatography are a combination of ion-pairing, trap and liquid–liquid partitioning chromatography. Typical mobile phases must be water-immiscible and include chloroform, methylene chloride, diethyl ether, light petroleum and toluene, among others. Ion-pairing and related column chromatography methodology, as described by Doyle and Levine [45,46], have been utilized extensively for the resolution of legitimate pharmaceutical drug mixtures for quantitation by ultraviolet spectroscopy. Forensic chemists have adapted this methodology for the isolation of alkaloidal impurities and manufacturing by-products from illicit cocaine and in the isola-

tion of alkaloids from coca leaf and other coca alkaloids [1,35,36].

In liquid–solid adsorption column chromatography, the stationary phase consists of activated adsorbents such as alumina, silica gel and Florisil; mobile phases are organic and vary widely in polarity. Typical mobile phases include chloroform, acetone, hexane, diethyl ether, methylene chloride, methanol and acetonitrile, among others. Separations are based, in part, on the relative polarities, solubility characteristics and/or molecular mass of the components in the drug matrix and upon the level of activity of adsorbent and polarity of mobile phase. The separation mechanisms are roughly analogous to those occurring in flat-bed chromatography, *e.g.* thin-layer chromatography, except in adsorption column chromatography the conditions are usually anhydrous. In fact, some types of adsorption column chromatography might be viewed as thin-layer chromatography in a column. A major advantage of this type of column chromatography, as was the case with Celite column chromatography, is the capability of fraction collection and the ease of varying the polarity of the mobile phase. These advantages have proven useful both in the isolation of manufacturing impurities in illicit, refined cocaine [14] and in the separation isolation of trace-level alkaloids from the bulk cocaine matrix in coca leaves [33,35]. A disadvantage that has been associated with this type of chromatography is a lack of reproducibility between different manufacturing batches of a given adsorbent.

In some instances liquid–liquid partition and liquid–solid adsorption column chromatography are used for gross separations, as a prelude to more refined isolation using higher-resolution techniques such a preparative HPLC.

4.2. Gas chromatography

Gas chromatography (GC) has been the most commonly used technique for generating chromatographic profiles of drug manufacturing impurities and by-products. This chromatography has been referred to as "impurity signature profiles", "geographic signature profiles" or "sample comparison profiles", among other names. Impurity signature methodology developed in the early-to-mid-1970s made use of packed columns for GC analyses. However, given the complex matrices associated with drug manufacturing impurities, packed columns often did not provide sufficient resolution. With the advent of fused-silica capillary columns, a marked improvement was realized in column efficiency and handling, providing the forensic chemist with a powerful analytical tool. Thus, since about 1980 the literature has seen a marked increase in reported methodology using cGC in the development of impurity signature profiles.

A plethora of capillary columns are now commercially available for use in the GC analyses of complex matrices. Furthermore, column selection has been greatly simplified with the advent of fused-silica capillary columns. A considerable body of experience in illicit drug analyses and in the characterization of alkaloidal impurities and manufacturing by-products indicate that columns of 15 m and 30 m in length, with an inner diameter of 0.25 mm, are suitable for most applications. For method development, column substrates covering a wide range of polarities have been investigated. Three of the most common are commercially available (J & W Scientific) DB-1 (non-polar), DB-5 (moderately polar) and DB-1701 (polar), all at a film thickness of 0.25 μm.

Several detection methods have been used in conjunction with the GC generation of impurity signature profiles. The most commonly used is flame-ionization detection (FID). It has sufficient sensitivity and dynamic range for the detection and determination of numerous drug manufacturing impurities and by-products. Typically, the on-column detection of nanogram levels of impurities is possible using FID. Other positive features of FID are its universal response to organic compounds and its ruggedness.

For the determination of impurities present at ultra-trace levels, FID, however, does not possess sufficient sensitivity. For these applications the use of electron-capture detection (ECD) may be warranted. When used with cGC, ECD is capable of routinely providing on-column detec-

tion levels in the low picogram and sometimes femtogram range for highly electrophilic compounds. Methodology using cGC–ECD generally requires a derivatization step that introduces an electrophilic group, such as a heptafluorobutyryl moiety or other halogenated group, into the impurity's structure [31].

Nitrogen–phosphorous detection (NPD) has been used sparingly in the cGC analysis of manufacturing impurities. For compounds containing one nitrogen atom, NPD exhibits a modest enhancement in sensitivity when compared to FID. However, it is markedly less sensitive compared to the ECD analysis of appropriately derivatized compounds. The less-than-rugged characteristic of NPD has probably limited its widespread use in signature analyses. Furthermore, the injection of certain solvents, such as silylating reagents, preclude the use of NPD. Such injections would coat the rubidium source, diminishing the concentration of this element in the flame plasma. An obvious advantage of NPD is its selectivity towards nitrogenous compounds.

When a mass spectrometer is used as GC detector, two primary advantages are realized. First, and clearly most significant, is the potential to structurally characterize the processing impurities and by-products. Although impurity signature profiles for geographic origin or sample comparison determinations can be accomplished without identification of all chromatographic components, their full structural characterization is desirable. Only in this manner can one fully rationalize the presence of impurities/by-products and discount their presence as artifacts. Second, the use of a mass spectrometer allows the incorporation of deuterated analogues of processing impurities as internal standards; such deuterated analogues are generally recognized as the ideal internal standards for the optimization of quantitative results and reproducibility.

4.3. High-performance liquid chromatography

The second most common technique for producing drug impurity signature profiles is HPLC. Because of its lower column efficiencies, HPLC is at a disadvantage, *vis-a-vis* cGC, in the analysis of the more complex impurity matrices. However, this limitation can be partly overcome by the use of multiple detectors. In terms of on-column detection levels, GC systems, in general, are more sensitive than those used in HPLC. This disadvantage can be partially compensated for by the fact that HPLC systems can accommodate much larger sample injections. A major attribute of HPLC is that compounds that do not chromatograph by GC because of their polarity, low volatility, or high molecular mass, are often amenable to HPLC analyses. This is perhaps best illustrated by considering the isomeric truxillines, which are alkaloids present in the coca leaf and in illicit refined cocaine samples. Having a molecular mass of 658, direct GC analysis of these alkaloids for analytical purposes is difficult because of thermal degradation in the injection port. However, direct chromatography of intact truxillines in illicit refined cocaine samples using HPLC has been successful and recently reported [15,21]. There is also some evidence to suggest that there are other high-molecular-mass alkaloids in coca leaf, heretofore unreported, that could be more suitable to HPLC rather than GC analysis.

A survey of the literature revealed that reversed-phase chromatography was favored for the HPLC analyses of drug manufacturing impurities, and for drug analyses in general. For these analyses, commercial columns included HS-5 C_{18}, μBondapak C_{18}, LC-8, RP-8 Spheri 10 and LiChrosorb RP-2 [8,10,15,20,21,25]. Typical mobile phases were usually binary and tertiary solvent mixtures that included acetonitrile, methanol and aqueous buffers, among others.

HPLC detection systems have been discussed for use in forensic drug analyses [31,47] and include dual variable-wavelength UV, diode-array UV, fluorescence and electrochemical detectors [47]. An inherent advantage of these detectors is the capability of interfacing them serially [47]; this is not possible with most cGC detection systems, such as FID and NPD, which are organic-compound destructive. The most commonly used detector for the analysis of cocaine and its manufacturing impurities/by-products are UV and diode-array UV detectors.

The latter detection system has the advantage of being able to monitor multiple wavelengths simultaneously, and in real time, thus providing some structural information.

The reported interfacing of HPLC with mass spectrometry (MS) for use in forensic drug analyses has been a rare occurrence. A major reason is that the majority of illicit drugs submitted for routine analyses are readily characterized by cGC–MS using electron ionization. Furthermore, if HPLC is interfaced with thermospray (TSP) MS, few data beyond molecular mass are obtained. Nonetheless, the use of TSP-MS has been reported for the HPLC analyses of a number of drugs of forensic interest [48,49]. Although no literature references were found for the application of TSP-MS in the analysis of drug manufacturing impurities, this technique holds promise for such. This is especially so for drugs that could be contaminated with high-molecular-mass compounds ($M_r > 600$) which are not amenable to cGC–MS analyses, especially those derived from natural products.

4.4. Micellar electrokinetic capillary chromatography

A new technique that appears very promising for forensic drug analyses is micellar electrokinetic capillary chromatography (MECC). First described in 1984 [50,51], MECC is a subclass of CE and offers significantly greater efficiency, selectivity, peak symmetry and speed, compared to HPLC. Detectors used in MECC include UV, fluorescence and electrochemical. The advantages of MECC over HPLC, *vis-a-vis* forensic drug analyses, have been described recently [52,53]. In those reports it was shown that drugs that chromatographed poorly by HPLC, or not at all by GC, exhibited good chromatographic behavior using MECC. The applicability of MECC for generating impurity signature profiles of illicit drugs such as cocaine and heroin is currently being investigated. As with TSP-MS the feasibility of determining high-molecular-mass impurities is good, as MECC has been previously reported for the analysis of nucleosides, peptides, porphyrins and antibiotics. A recognized deficiency of MECC is sensitivity, which is below that for HPLC–UV [52,54]. This could limit the use of MECC in the analysis of ultra-trace levels of manufacturing impurities.

5. Chemical derivatization

An invaluable adjunct of chromatographic analyses is chemical derivatization (ChD). This technique has played an integral role in the development of impurity signature profiles for manufacturing impurities/by-products in illicit cocaine, as well as heroin [31]. It is not the intent of this review to discuss ChD in detail, as it has been covered in-depth for general applications [55] and, specifically, for forensic drug analyses [31]. Briefly, the major reasons for the application of ChD in chromatographic analyses are to improve chromatographic behavior of target compounds and/or to enhance their detectability. Drugs and manufacturing impurities/by-products most likely to undergo derivatization have functional groups with labile protons, including RNH_2, R_1R_2NH,–OH,–COOH and–SH. There have also been reports of very unusual high-yield acylation at carbon sites in some drugs and their manufacturing impurities [31]. The most common derivatization reactions pertaining to illicit drugs include silylation, acylation and alkylation. Although ChD has been applied sparingly in routine illicit drug analyses, its greater impact is upon the analysis of manufacturing impurities, especially in lowering their minimum detectable levels. Although ChD is more widely used in cGC analyses, it can also be useful in HPLC, especially for the introduction of chromophoric or fluorophoric groups into the drugs's structure for enhanced UV or fluorescence detection.

6. Artifact formation

During the legitimate in-depth analyses of illicit drugs and natural products, especially for intelligence purposes, the chemist should be aware of the inadvertant creation of artifactual compounds during the work-up of samples. This is especially applicable to trace analyses. These artifacts are usually formed by the interaction of

bona fide alkaloids in the plant or refined drug with chemicals, solvents, and impurities in solvents as well as by the application of heat during the alkaloid isolation process. These interactions can result in epimerization, hydrolysis and N-demethylation of plant alkaloids. Thus, in coca leaf analyses using conventional extraction techniques, the detection of cocaine hydrolysis products, *e.g.*, benzoylecgonine and ecgonine, should be viewed cautiously. Likewise, the presence of trace levels of N-norcocaine in a coca leaf extract may be the result of the peroxide-assisted N-demethylation of cocaine *vs.* its presence as a *bona fide* alkaloid. Peroxides are common in some solvents, especially diethyl ether. Epimerization at the C-2 site in the tropane moiety can occur if the alkaloid work-up includes the use of a strong base. Finally, as will be discussed in a subsequent section of this review, it is suspected that the reported presence of low levels of hygrine in coca may in fact be due to the degradation of cuscohygrine during coca leaf analysis. From the foregoing, it is apparent that, in some cases, artifact formation during the legitimate analyses of coca and cocaine closely mirrors the creation of by-products during the clandestine manufacture of refined cocaine from coca leaf.

Artifact formation can also occur during chromatographic analyses of an alkaloid extract, especially if GC is used. For example, ecgonidine methyl ester has been incorrectly reported as a *bona fide* coca leaf alkaloid. Actually, this compound is an artifact formed in the heated injection port of the gas chromatograph, from the thermal degradation of cocaine [6] and/or the truxillines [14]. In a recent GC study, it was demonstrated that cocaine, in the presence of base, can undergo hydrolysis, transesterification and epimerization in the injection port [56].

7. Chromatographic analyses of alkaloids in coca leaf

From a forensic perspective, the in-depth analyses of alkaloids in the coca leaf are important, because these endogenous compounds, and the by-products derived from them, appear in illicit cocaine samples. As discussed in the introduction of this review, such data can be used for tactical and strategic law enforcement purposes. Furthermore, the structural elucidation of these alkaloids in the leaf allows for their more facile characterization in illicit, refined cocaine; this is important because, in some instances, these alkaloids experience a marked diminution in the processing of leaf to refined cocaine. Finally, the only unequivocal method for determining if an impurity detected in illicit cocaine is a *bona fide* leaf alkaloid, a manufacturing by-product, or both, is after careful examination of the alkaloid content of the leaf.

Virtually all illicit cocaine is derived from South American coca leaf. There are four recognized coca varieties derived from two species and a single genus, that contain significant levels of cocaine [57,58]. These are *Erythroxylum coca* var. *coca* (ECVC), *Erythroxylum coca* var. *ipadu* (ECVI), *Erythroxylum novogranatense* var. *novogranatense* (ENVN) and *Erythroxylum novogranatense* var. *truxillense* (ENVT). Historically, ECVC is the cultivar that has been used for the manufacture of illicit cocaine. This is doubtless because this variety grows over a much larger geographic area than do the other cultivars. Also, the cocaine content of ECVC is similar to or higher than in the other varieties. Finally, varieties such as *novogranatense* and *truxillense* contain greater levels of other alkaloids that are difficult to remove from cocaine, via current illicit production methods, thus contaminating the refined cocaine product.

Most South American coca leaf is cultivated on the eastern slopes and valleys of the Andes mountains ranging from Colombia to Bolivia. Most of the ECVC cultivated for illicit cocaine manufacture is done so in the Upper Huallaga Valley of Peru and the Chapare region of Bolivia. The cocaine content of this leaf is usually in the 0.5–0.8% range (relative to dry mass leaf) [59]. The processing of ECVC coca to yield refined cocaine has been described adequately elsewhere [60,61] and will not be discussed in detail here. Occasional reference to this methodology will be necessary, however, in relating components of the coca leaf to alkaloidal

impurities and manufacturing by-products in illicit refined cocaine.

In addition to cocaine, coca leaves contain numerous other alkaloids, including among others *cis*- and *trans*-cinnamoylcocaine, tropacocaine, the isomeric truxillines, cuscohygrine and hygrine [38]. The structures for some of these alkaloids are seen in Figs. 1, 4 and 7. Until

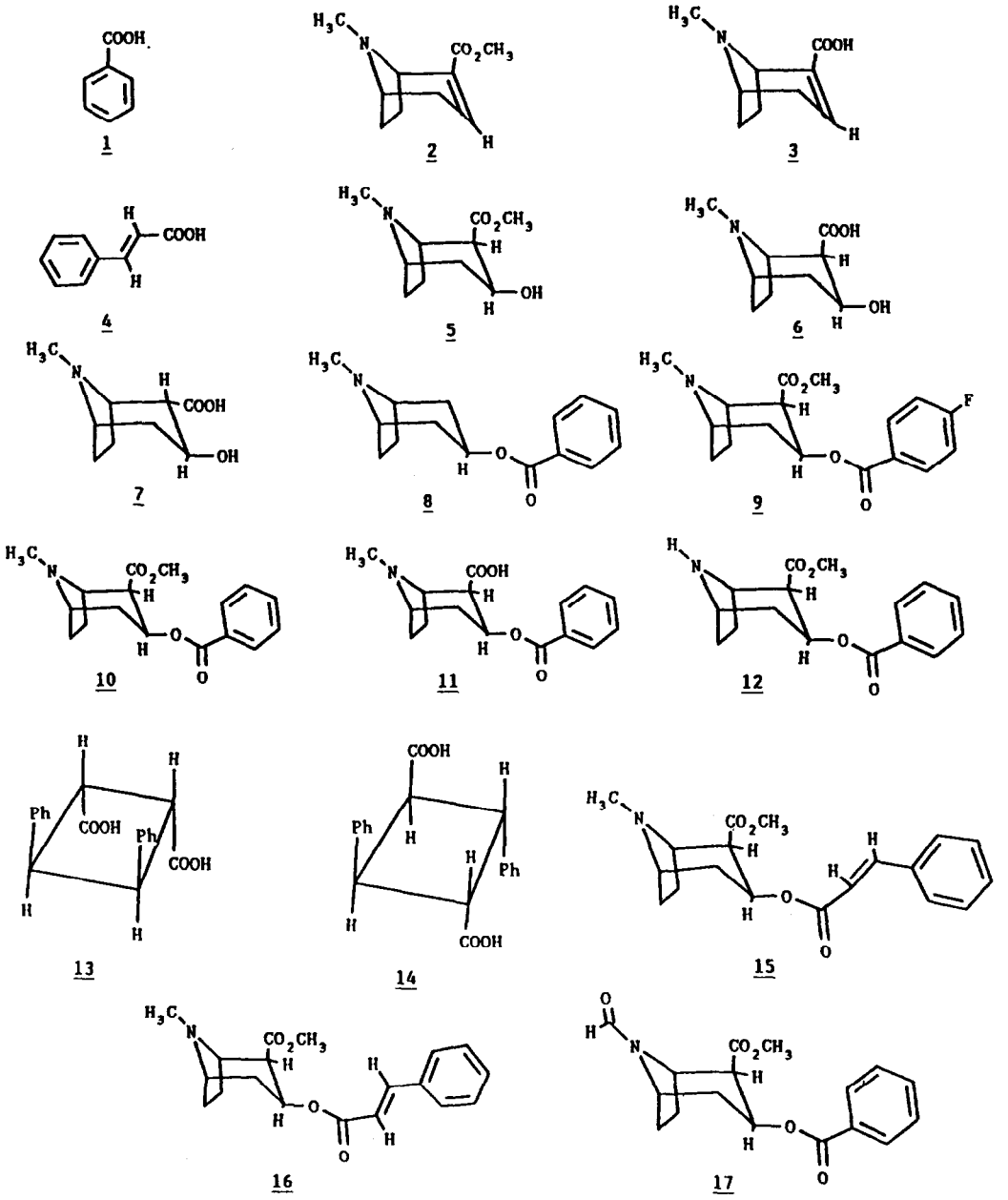


Fig. 1. Structures of some alkaloids in coca leaf and alkaloidal impurities and manufacturing by-products in illicit refined cocaine: 1 = benzoic acid; 2 = ecgonidine methyl ester; 3 = ecgonidine; 4 = *trans*-cinnamic acid; 5 = ecgonine methyl ester; 6 = ecgonine; 7 = pseudoecgonine; 8 = tropacocaine; 9 = *p*-fluorococaine internal standard; 10 = cocaine; 11 = benzoylecgonine; 12 = N-norcocaine; 13 = β-truxinic acid; 14 = α-truxillic acid; 15 = *cis*-cinnamoylcocaine; 16 = *trans*-cinnamoylcocaine; 17 = N-formylnorcocaine. From ref. 26.

recently, the only quantitative data available for South American ECVC and ENVN coca leaf alkaloids, as well as alkaloids from greenhouse-cultivated coca plants, pertained primarily to cocaine and *cis*- and *trans*-cinnamoylcocaine. Recently, however, quantitative data have been reported for other coca alkaloids, including the isomeric truxillines, tropacocaine, cuscohygrine and hygrine [34,35]. This was accomplished not only for South American ECVC and ENVN, but also for greenhouse- and non-South American tropical-cultivated ECVC, ENVN and/or ENVT.

7.1. Cocaine and the cinnamoylcocaines

In one of the early studies of forensic interest, Moore [1] applied ion-pairing column chromatography for the partial resolution of *cis*- and *trans*-cinnamoylcocaine, tropacocaine and cocaine in a coca leaf extract. Those alkaloids were eluted as hydrochloride ion pairs from a dilute hydrochloric acid/Celite column with water-saturated chloroform. This chromatography, seen in Fig. 2, provided sufficient resolution to allow, for the first time, structural characterization of the isomeric cinnamoylcocaines by UV, IR, MS and ^{1}H NMR spectroscopy. No quantitative data for the cinnamoylcocaines was provided in that study.

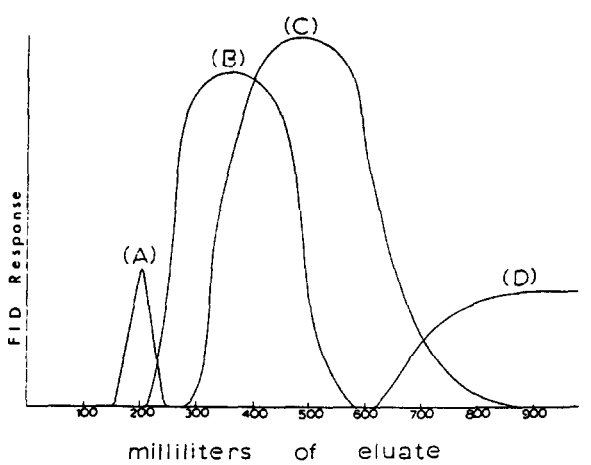


Fig. 2. Column partition chromatographic (ion-pairing) elution pattern of (A) tropacocaine, (B) *trans*-cinnamoylcocaine, (C) *cis*-cinnamoylcocaine and (D) cocaine. From ref. 1.

There have been a number of subsequent reports describing the quantitation of cocaine and *cis*- and *trans*-cinnamoylcocaine in coca leaves [58,62–67]. In one of the most significant of these, Plowman and Rivier [58] reported the cocaine and *cis*- and *trans*-cinnamoylcocaine content of ECVC, ECVI, ENVN and ENVT cultivated in Colombia, Ecuador, Bolivia and Peru. Using a stable-isotope dilution method and GC–MS with selected-ion monitoring, they reported a mean cocaine content of 0.63% and a mean *cis*- + *trans*-cinnamoylcocaine content of 10.8% for ECVC leaf (relative to cocaine). The *cis*-/*trans*-ratio for the ECVC coca had a mean value of 2.7. Table 1 summarizes the data for all four coca varieties.

In a packed column GC–FID study of ECVC leaves from Peru, Turner *et al.* [62] determined the cocaine content of samples collected from three disparate geographical regions. They reported cocaine levels in air-dried leaf of 0.60, 0.57 and 0.60% from leaves harvested in Cuzco, Trujillo and Tingo Maria, respectively. In a follow-up study by the same group [63], Peruvian ECVC leaves from three locations were analyzed for both cocaine and cinnamoylcocaine content. Differences were observed in the cinnamoylcocaine levels from each site and the relative ratios of total cinnamoylcocaines to cocaine varied with sample origin. In this latter study, coca leaf extracts were also examined by GC–NPD and GC–MS.

In an investigation using packed-column GC–MS, Holmstedt *et al.* [64] examined 13 South American species of *Erythroxylum* for cocaine content. Cocaine was found in only ECVC, ENVT and ENVN leaf from Bolivia, Peru and Colombia. The average dried-leaf cocaine content of 12 ECVC samples from Peru was 0.73% while two samples of Bolivian ECVC leaf had cocaine levels of 0.70 and 0.74%. Leaves of 10 ENVN samples collected in Colombia varied in cocaine content from 0.17 to 0.76%, with an average of 0.47%. A single sample of Peruvian ENVT leaf had a cocaine content of 0.71%. The quantitative accuracy of the foregoing results was

Table 1
Summary of cocaine and cinnamoylcocaine content of dried leaves of the cultivated cocas *E. coca* and *E. novogranatense*

	Total							
	Cocaine		Cinnamoylcocaines		*cis*		*trans*	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
E. coca var. coca (30 samples)	0.23–0.96	0.63	0.0011–0.532	0.068	0–0.44	0.05	0–0.11	0.0183
E. coca var. ipadu (6 samples)	0.11–0.41	0.25	0–0.0084	0.005	0–0.008	0.005	0	0.0
E. novogranatense var. novogranatense (3 samples)	0.55–0.93	0.77	0.107–0.65	0.379	0.072–0.53	0.287	0.035–0.12	0.092
E. novogranatense var. truxillense (14 samples)	0.42–1.02	0.72	0–0.93	0.231	0–0.68	0.154	0–0.43	0.0775

Data reported as mg 100 mg^{-1} dry mass. From ref. 58.

enhanced by the use of a deuterated cocaine standard.

Amazonian coca, *i.e.* ECVI, has been reported to have consistently lower cocaine content compared to Andean coca [58]. In a study of freshly air-dried leaves of Amazonian coca, the cocaine content ranged from only 0.11 to 0.39% [65] (authors' note: like South American ENVN and ENVT coca, leaves of ENVI are not believed to be a major factor in illicit cocaine production).

In a cGC–MS study by Rivier [66], using deuterated cocaine as an internal standard, the intra-plant variation of cocaine and the cinnamoylcocaines in coca leaves was found to be significant. It was pointed out that the variation of alkaloid levels in coca leaf can depend upon environmental conditions and leaf age. It was concluded that the variation of the alkaloid content in individual leaves was too great to allow the use of the ratio of cocaine to the cinnamoylcocaines as a taxonomic marker [authors' note: Rivier's study should encourage others to use sufficient sample size in the determination of coca leaf alkaloids, so as to render intra-and inter-plant (from the same plot) variations a non-factor when evaluating data].

In unique, non-chromatographic methods by Youssefi *et al.* [67] and Cooks *et al.* [68], cocaine/cinnamoylcocaine ratios were determined in individual coca leaves using mass-analyzed ion kinetic energy spectrometry (MIKES). They determined that coca plants from different geographic regions could be distinguished on the basis of alkaloid content. They also confirmed the findings of Rivier [66] relating to the significant intra-plant leaf variation *vis-a-vis* the cocaine/cinnamoylcocaine ratios. Among the purported advantages of MIKES were small sample size (1 mg), reduced analysis time and minimization of sample preparation, in particular the avoidance of solvent extractions. The latter is especially advantageous because of the problem of artifact formation during solvent extractions of natural products.

In a coca leaf method described by Solon and Sperling [69], and in current use by the US Drug Enforcement Administration, cocaine was quantitated by cGC–FID after its extraction from the leaf with warm (75°C) methanol. As will be discussed subsequently, this method, though not touted as such, also appeared suitable for the determination of *cis*- and *trans*-cinnamoylcocaine [35].

7.2. Cocaine, cinnamoylcocaines, tropacocaine, truxillines, ecgonine methyl ester, cuscohygrine and hygrine (see Figs. 1, 4 and 7)

In the most in-depth coca leaf/alkaloid study to date, Moore *et al.* [35] recently reported the quantitative alkaloid analyses of cocaine-bearing plants from the field in South America, a greenhouse in the USA and from a tropical site other than South America. The samples examined included ECVC and ENVN from Bolivia, Peru, Ecuador and Colombia, greenhouse-cultivated ECVC, ENVN and ENVT and non-South American tropically grown ECVC and ENVN cultivars. In that study the rationale behind the analyses of greenhouse cultivars and coca leaf other than ECVC was three-fold. First, such in-depth data had never been previously reported. Second, it was of forensic interest to know whether coca leaf of a species/variety other than ECVC yielded similar or markedly different alkaloidal profiles. Third, it was hoped that uncharacterized trace-level alkaloids in ECVC might be present at significantly higher levels in greenhouse-cultivated coca leaves, allowing for their more facile isolation and structural elucidation.

In the methodology referenced above [35], dried coca leaves were reduced to a powder, basified and the alkaloids extracted in high yield from the leaf using water-saturated toluene. Cocaine and other alkaloids were isolated from interfering leaf components in the toluene extract by retaining the former on a dilute sulfuric acid/Celite column. After the alkaloids were liberated from the column, they were quantitated using cGC–FID and cGC–ECD. In addition to the determination of cocaine and *cis*- and *trans*-cinnamoylcocaine, the quantitation of the alkaloids ecgonine methyl ester, tropacocaine, the isomeric truxillines, cuscohygrine and hygrine were reported for the first time. The accuracy and reproducibility of this methodology were enhanced by the use of structurally related internal standards [35]. The quantitative results for ecgonine methyl ester, cuscohygrine, tropacocaine, cocaine and *cis*- and *trans*-cinnamoylcocaine in South American, greenhouse and non-South American tropical-cultivated coca are found in Tables 2 and 3. The cGC–FID chromatogram for the determination of selected alkaloids in greenhouse-cultivated ENVN coca is illustrated in Fig. 3. A brief review of the results from the Moore *et al.* method is given below.

7.2.1. Cocaine and the cinnamoylcocaines

The cocaine and cinnamoylcocaine content for Bolivian and Peruvian ECVC leaf (Table 2) were in reasonable agreement with other studies. The much higher cinnamoylcocaine content for Colombian leaf was believed related to its taxonomy, which was suspected to be ENVN coca, and not due to environmental conditions. The markedly higher cinnamoylcocaine levels for greenhouse- and tropical-cultivated ENVN and ENVT coca *vs.* ECVC (Table 3) were also due to taxonomic differences. When the *trans*/*cis*-cinnamoylcocaine ratios for the greenhouse cul-

Table 2
Quantitative results for cocaine and other coca alkaloids in South American cultivated coca leaves (see ref. 35)

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

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.

Table 3
Quantitative results for cocaine and other coca alkaloids in greenhouse- and tropical-cultivated coca leaves (see ref. 35)

Alkaloid	ECVC (G)[a]	ENVT (G)[b]	ENVN (G)[c]	ENVN (T)[d]	ECVC (T)[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

All cocaine results are % (w/w) and are calculated relative to dry leaf. Results for all alkaloids, excepting cocaine, are % (w/w) and are calculated relative to cocaine content.
[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 benzoyltropine, an epimer of tropacocaine, at a level of 1.1% (relative to cocaine content).

tivars in Table 3 were compared with the same ratios for South American coca in Table 2, it was seen that the ratios of the former were significantly higher. The authors attributed this to the fact that less UV light is available in the greenhouse environment to convert the *trans* isomer to its *cis* counterpart [70].

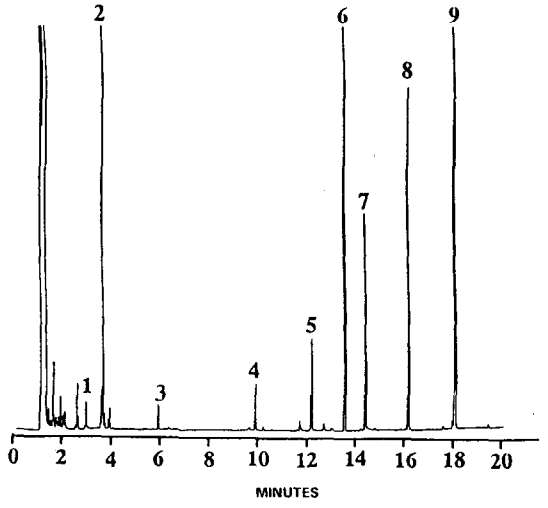


Fig. 3. The cGC–FID chromatogram of major coca alkaloids isolated and determined in greenhouse-cultivated *E. novogranatense* var. *novogranatense*. Peaks: 1 = ecgonidine methyl ester; 2 = ecgonine methyl ester; 3 = cuscohygrine; 4 = tropacocaine; 5 = 1-hydroxytropacocaine; 6 = cocaine; 7 = cocaethylene internal standard; 8 = *cis*-cinnamoylcocaine; 9 = *trans*-cinnamoylcocaine (see ref. 35).

7.2.2. Tropacocaine

With one exception, the tropacocaine levels seen in Tables 2 and 3 were unremarkable. As was the case for the cinnamoylcocaines, the tropacocaine content for the ECVC coca was markedly lower than that of ENVN and ENVT leaf, again believed due to differences in taxonomy. One of the most interesting result reported by the authors was for the greenhouse-cultivated ECVC leaf. As seen in Table 3, the epimer of tropacocaine, namely benzoyltropine, was 3 × higher in concentration. This compares with Peruvian and Bolivian coca leaf in which tropacocaine is readily detectable, but benzoyltropine, when present, is at significantly reduced levels

7.2.3. Truxillines

The coca leaf extract described above also contained the isomeric truxillines, seen in Fig. 4. These alkaloids had been previously characterized in illicit refined cocaine samples [14,18]. After isolation from the leaf, the truxillines were reduced with lithium aluminum hydride to their respective diols, which were then derivatized with heptafluorobutyric anhydride (HFBA) to

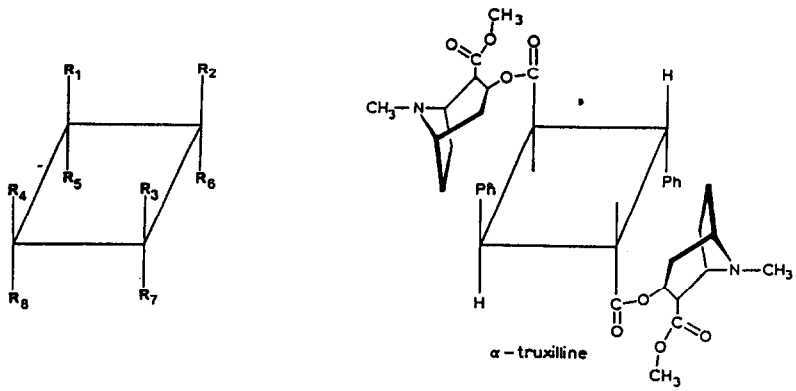


Fig. 4. Structures for the isomeric truxillines. (Left) General structure. (1) α, $R_1 = R_7$ = methyl ecgonine ester, $R_4 = R_6$ = phenyl, $R_2 = R_3 = R_5 = R_8 = H$; (2) β, $R_5 = R_6$ = methyl ecgonine ester, $R_3 = R_4$ = phenyl, $R_1 = R_2 = R_7 = R_8 = H$; (3) δ, $R_2 = R_5$ = methyl ecgonine ester, $R_4 = R_7$ = phenyl, $R_1 = R_3 = R_6 = R_8 = H$; (4) ϵ, $R_5 = R_7$ = methyl ecgonine ester, $R_2 = R_4$ = phenyl, $R_1 = R_3 = R_6 = R_8$; (5) μ, $R_1 = R_6$ = methyl ecgonine ester, $R_4 = R_7$ = phenyl, $R_2 = R_3 = R_5 = R_8 = H$; (6) γ, $R_1 = R_3$ = methyl ecgonine ester, $R_4 = R_6$ = phenyl, $R_2 = R_5 = R_7 = R_8 = H$; (7) neo, $R_2 = R_5$ = methyl ecgonine ester, $R_3 = R_4$ = phenyl, $R_1 = R_6 = R_7 = R_8 = H$; (8) ζ, $R_5 = R_6$ = methyl ecgonine ester, $R_4 = R_7$ = phenyl, $R_1 = R_2 = R_3 = R_8 = H$; (9) *epi*, $R_1 = R_7$ = methyl ecgonine ester, $R_2 = R_4$ = phenyl, $R_3 = R_5 = R_6 = R_8 = H$; (10) *peri*, $R_1 = R_3$ = methyl ecgonine ester, $R_2 = R_4$ = phenyl, $R_5 = R_6 = R_7 = R_8 = H$; (11) ω, $R_1 = R_2$ = methyl ecgonine ester, $R_3 = R_4$ = phenyl, $R_5 = R_6 = R_7 = R_8 = H$. (Right) Structure of α-truxilline, Ph = phenyl (see refs. 14, 18 and 35).

yield ECD-sensitive diheptafluorobutyryl (di-HFB) derivatives. This reaction is shown in Fig. 5. The di-HFB derivatives were determined by cGC–ECD using a 30-m fused-silica capillary column coated with DB-1701. This chromatography is illustrated in Fig. 6 for a coca leaf sample from Peru. The accuracy and reproducibility of the truxilline quantitative data were enhanced by the use of a structurally related internal standard, namely, μ-truxinic acid. The quantitative results for truxillines in South American coca are presented in Table 4. The much higher levels of truxillines in the Colombian sample was again believed due to differences in plant taxonomy. Truxilline data from the greenhouse coca were not reported.

7.2.4. Ecgonine methyl ester

The quantitative levels of this alkaloid were reported only for the greenhouse and non-South American tropical coca cultivars. As seen in Table 3, ecgonine methyl ester was present in those leaves at unexpectedly high levels (relative to cocaine). Whereas the presence of this compound in illicit refined cocaine samples is attributed to cocaine hydrolysis, the values for ecgonine methyl ester in Table 3 were due to *bona fide* alkaloid, *i.e.*, present in the leaf itself.

7.2.5. Cuscohygrine and hygrine

Unlike most coca alkaloids, cuscohygrine and hygrine, seen in Fig. 7, do not contain the tropane moiety. These N-methylpyrrolidine alkaloids, especially cuscohygrine, vary widely in quantitative levels between cultivars. There has also been some evidence that hygrine may be created as an artifact. As seen in Tables 2 and 3, cuscohygrine was present at the highest concentrations in all four of the ECVC cultivars, with an average of 58% (relative to cocaine). Conversely, the cuscohygrine content of the ENVN and ENVT leaf (Tables 2 and 3) averaged only 13%. It is not clear if these wide differences were also related to leaf taxonomy.

Hygrine has been reported previously as an alkaloidal component in South American coca [38]. In the Moore *et al.* study [35], the apparent hygrine content of coca leaf from Peru, Bolivia, Ecuador and Colombia was reported to be 1.4, 2.3, 4.2 and 24%, respectively (relative to cocaine). It was also observed by the authors that, over the course of several weeks, the

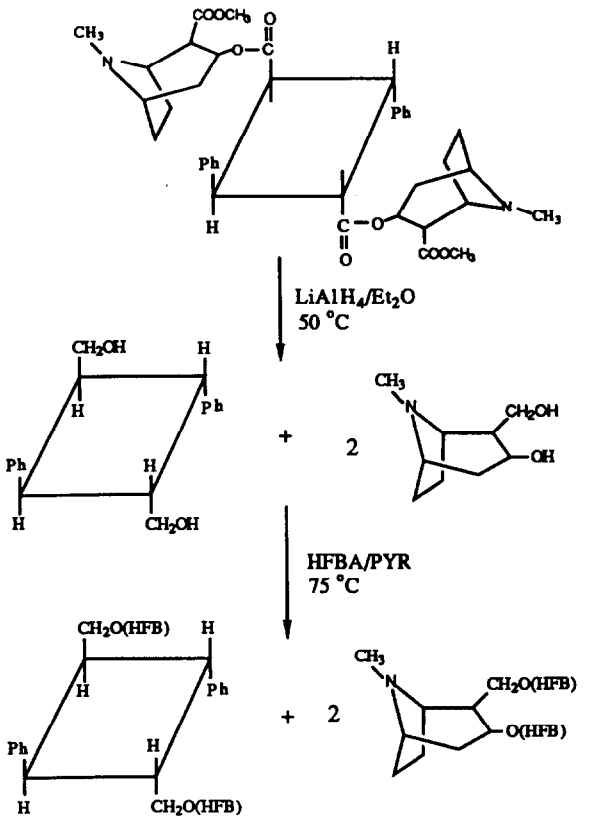


Fig. 5. Lithium aluminum hydride reduction of α-truxilline and derivatization with heptafluorobutyric anhydride (see refs. 14, 18 and 35).

cuscohygrine content of powdered Peruvian and Bolivian leaf, stored at room temperature, gradually decreased while, concomitantly, their hygrine content increased. This suggested that the hygrine content of that leaf might be in part, or in whole, artifactual. There was also suspicion that during the work-up of cuscohygrine, its partial degradation to hygrine may occur.

8. New coca leaf alkaloids

8.1. Hydroxycocaines

In a recent study by Moore and Cooper [33], 4–7 hydroxycocaines were presumptively identified at trace levels in illicit refined cocaine samples. Mass spectral analysis of these alkaloids

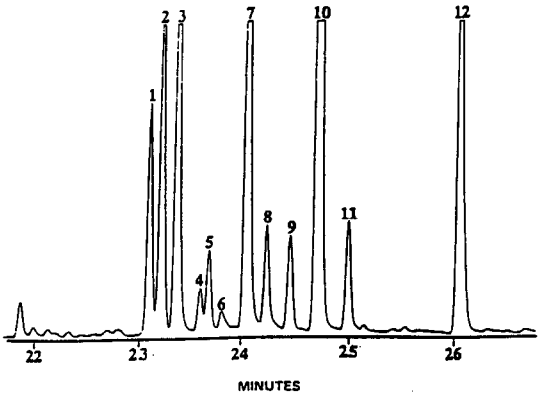


Fig. 6. The cGC–ECD chromatogram (30 m × 0.25 mm I.D. DB-1701) of the truxillines isolated from Peruvian coca leaf. Pertinent chromatographic peaks are the result of lithium aluminum hydride reduction of the truxilline followed by derivatization with heptafluorobutyric anhydride to yield a di-O-heptafluorobutyryl derivative. Peak identification of truxilline isomers: 1 = ϵ-; 2 = δ-; 3 = β-; 4 = *peri*-; 5 = neo-; 6 = *epi*-; 7 = α-; 8 = ω-; 9 = γ-; 10 = μ- (added as internal standard); 11 = ζ-; 12 = heneicosanol internal standard (chromatographed as O-HFB derivative) (see Figs. 4 and 5 and refs. 14, 18 and 35).

Table 4
Isomeric truxilline content of South American cultivated coca leaves (see ref. 35)

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

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

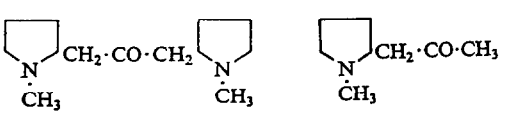


Fig. 7. Structures for cuscohygrine (left) and hygrine (right).

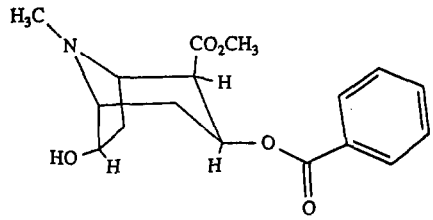


Fig. 8. Structure for 6-hydroxycocaine (see refs. 33 and 35).

suggested hydroxy substitution on the tropane ring. A preliminary investigation of South American coca leaf indicated the presence of at least four of the hydroxycocaines at very low levels [33,71]. One of these, believed to be 6-hydroxycocaine, is illustrated in Fig. 8.

8.2. Trimethoxy analogues of cocaine, cinnamoylcocaines and tropacocaine

In a modification of the Moore *et al.* method [35] described above, Casale and Moore [36] used toluene extraction of basified South American coca leaf, followed by trap and ion-pairing column chromatography, to isolate four previously unknown coca alkaloids in South American coca leaf. The structural characterization of 3′,4′,5′-trimethoxycocaine, the *cis* and *trans* isomers of 3′,4′,5′-trimethoxycinnamoylcocaine and 3′,4′,5′-trimethoxytropacocaine, all seen in Fig. 9, was accomplished by the comparison of their electron ionization mass spectra with synthesized standards. Quantitative data were provided by cGC–FID; the accuracy of this method was enhanced by using 3′,4′,5′-trimethoxycocaethylene as a structurally related internal standard. The quantitative data for a limited number of coca leaf samples from Brazil, Colombia, Bolivia and Peru are reported in Table 5. It was interesting to note that the total trimethoxycinnamoylcocaine content for the Peruvian and Bolivian leaf samples was 4–5 × higher than their respective levels of trimethoxy-

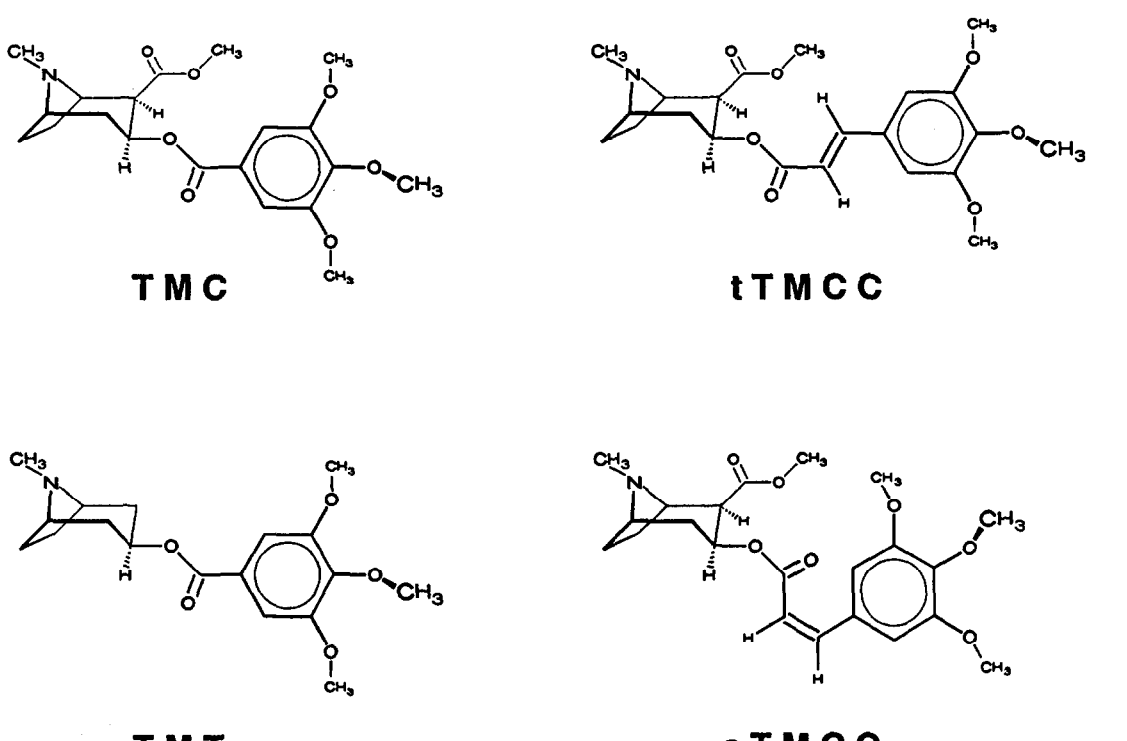


Fig. 9. Structures for some trimethoxy-substituted tropane alkaloids in South American coca: TMC = 3′,4′,5′-trimethoxycocaine; tTMCC = 3′,4′,5′-trimethoxy-*trans*-cinnamoylcocaine; cTMCC = 3′,4′,5′-trimethoxy-*cis*-cinnamoylcocaine; TMT = 3′,4′,5′-trimethoxytropacocaine. From ref. 36.

Table 5
Trimethoxy-substituted alkaloid content in South American coca leaf

Country	TMT[a]	TMC[b]	cTMCC[c]	tTMCC[d]
Brazil	–	1.12	–	0.15
Colombia	–	–	–	–
Bolivia-1	–	0.14	0.13	0.74
Bolivia-2	–	0.22	0.11	0.95
Peru	0.18	0.24	0.16	0.83

Results calculated using 3′,4′,5′-trimethoxyethylcocaine as the internal standard. All data are presented as % (w/w) relative to cocaine. From ref. 36.
[a] 3′,4′,5′-Trimethoxytropacocaine.
[b] 3′,4′,5′-Trimethoxycocaine.
[c] 3′,4′,5′-Trimethoxy-*cis*-cinnamoylcocaine.
[d] 3′,4′,5′-Trimethoxy-*trans*-cinnamoylcocaine.

cocaine. These data were contrasted by the cocaine content of leaf from Peru and Bolivia being 8–10 × greater than the cinnamoylcocaine levels. The authors of this study cautioned that many more samples needed to be examined before determining if this data are representative.

8.3. Other alkaloids

In addition to the coca leaf alkaloids described above, Moore *et al.* [35] reported the presumptive presence of many additional previously uncharacterized trace level alkaloids in Bolivian and Peruvian coca leaf. After the toluene extraction of basified and powdered coca leaves, the extract was subjected to trap and ion-pairing column chromatography as well as extractions with a pH 4 buffer. Additional resolution of the complex alkaloid mixture was provided by column chromatography on basic alumina using an elutropic series of chloroform, chloroform–acetone, acetone, acetone–methanol and methanol [71]. All fractions were subjected to trimethylsilylation followed by cGC–MS analyses. Of the 100 or so suspected alkaloids, more than half yielded mass spectra that exhibited fragment ions at m/z 82 and 182, indicating the presence of a carbomethoxytropane moiety, such as that found in cocaine. Furthermore, the majority of these compounds possessed derivatizable functional groups, an important consideration if sensitive cGC–ECD analysis of these alkaloids is contemplated. The individual levels of all of these new alkaloids were well below 0.1% (relative to cocaine).

9. In-depth analysis of illicit refined cocaine

The manufacture of illicit refined cocaine from South American coca leaf is a somewhat unsophisticated and crude, albeit effective, process. Although there are many clandestine cocaine laboratories throughout the coca-producing regions of South America, they all use methodology that is similar in their basic elements. Briefly, after harvesting, the coca leaves are basified with an inorganic salt and extracted with kerosene or alternatively, extracted directly with dilute sulfuric acid. The resulting extract is then subjected to additional liquid–liquid extractions, precipitations, a potassium permanganate oxidation step and, finally, conversion of cocaine base to cocaine hydrochloride. A more detailed account of the cocaine manufacturing process is discussed elsewhere [60,61].

Because of the unsophisticated nature of the cocaine manufacturing process, a multitude of trace-level alkaloidal impurities are present in illicit refined cocaine. Many of these impurities are *bona fide* alkaloids that cohabit the leaf with cocaine and are "carried through" the manufacturing process. In addition, and as discussed previously, cocaine samples are also contaminated with a variety of manufacturing by-products, *i.e.* compounds resulting from alkaloid degradation. As a result, the cocaine content of unadulterated samples rarely exceeds 98–99%. In most unadulterated samples, cocaine levels range from 80–97%. A review of published reports describing the presence of alkaloidal impurities and manufacturing by-products in illicit refined cocaine is presented below.

9.1. Alkaloidal impurities in illicit refined cocaine

A comparison of alkaloids in coca leaf and alkaloidal impurities that have been reported in

illicit refined cocaine is presented in Table 6. As seen, there is significant "carryover" of the tropane alkaloids from the leaf to refined cocaine during the manufacturing process. It is also apparent that some alkaloids are at least semi-quantitatively carried through to the final product while others experience a more marked decline in concentration (relative to cocaine) in proceeding from the leaf to refined cocaine.

9.1.1. Cinnamoylcocaines

First reported in illicit cocaine by Moore in 1973 [1], *cis*- and *trans*-cinnamoylcocaine (Fig. 1) are almost always found together when present in illicit refined cocaine samples. They are readily detected in the vast majority of cocaine samples by either GC–FID or HPLC–UV methodology. In the first study of its kind, Clark [4] used packed column GC–FID to quantitate and report the cinnamoylcocaine content of 40 cocaine samples. The *cis*- + *trans*-cinnamoylcocaine levels varied from less than 0.10% to 5.17%, with a mean content of 1.45% (relative to cocaine). The *cis*-/*trans*-ratio for 32 of the samples having quantifiable levels of the cinnamoylcocaines had a mean value of 1.4. Jane *et al.* [8] are believed to be one of the first to describe the HPLC–UV analysis of illicit cocaine samples. In that study, the cinnamoylcocaines were detected in only 126 cocaine samples out of

Table 6
Selected coca alkaloids detected in South American coca leaves and refined illicit cocaine

Alkaloid	Coca leaf	Refined cocaine	Comments[a]
cis-Cinnamoylcocaine	Yes	Yes	0–53% in leaf; 1.9%[b] in refined cocaine
trans-Cinnamoylcocaine	Yes	Yes	0–170% in leaf; 1.2%[b] in refined cocaine
Truxillines	Yes	Yes	2–60% in leaf; 2–13% in refined cocaine
Tropacocaine	Yes	Yes	0.2–5% in leaf; 0.02%[b] in refined cocaine
Ecgonine methyl ester	Yes	No	30–60% in leaf; < 3% in refined cocaine, due to cocaine hydrolysis?
Hydroxycocaines	Yes	Yes	4 detected in leaf, at < 0.01%; 4–7 detected in refined cocaine, at < 0.01%
3′,4′,5′-Trimethoxycocaine + 3′,4′,5′-trimethoxy-*cis*-cinnamoylcocaine + 3′,4′,5′-trimethoxy-*trans*-cinnamoylcocaine + 3′,4′,5′-trimethoxytropacocaine	Yes	Yes	0.1–1% in leaf; 0–0.1% in refined cocaine
Cuscohygrine	Yes	No	10–80% in leaf; not reported in refined cocaine
Hygrine	Yes	No	1–25% in leaf; not reported in refined cocaine

[a] All coca leaf % values are based upon the analyses of a limited number of samples. All % values are relative to cocaine content. All % results for coca leaf included the four major coca varieties (even though ECVC is the cultivar reportedly used most often in clandestine cocaine manufacture); all data were obtained from Tables 1-5 and 7 as well as refs. 26, 35, 36 and 76.
[b] Average of 4000 analyses (see ref. 26).

a total of 336 examined. This could have been due to the lesser sensitivity of HPLC–UV *vs.* GC–FID, or the implementation of a high attenuation during generation of the chromatographic profiles. Shortly after the study by Jane *et al.*, Noggle and Clark [10] also reported the detection of the cinnamoylcocaines using HPLC–UV methodology. Lurie *et al.* [15] used HPLC and photodiode-array UV detection for the analysis of the cinnamoylcocaines as well as other alkaloidal impurities and manufacturing by-products. Using packed-column GC–FID, Ensing *et al.* [30] reported the presence of the cinnamoylcocaines in about 85% of 71 cocaine samples examined. cGC–FID, cGC–MS and HPLC–UV were employed by LeBelle and co-workers [20,25] to provide quantitative data for the cinnamoylcocaines. The intra- and inter-sample variability of these alkaloids as well as their stability was also investigated.

In the most comprehensive study done to date, conducted from 1989 to 1992, Casale and Waggoner [26] determined the *cis*- and *trans*-cinnamoylcocaine content of about 4000 illicit refined cocaine seizures using cGC–FID. The data revealed that the cinnamoylcocaines were detected in $>95\%$ of samples and varied between about 0.1 and 9% (relative to cocaine), with a mean total cinnamoylcocaine level of 3.2%. As in the Clark study [4], the *cis* predominated over the *trans* isomer, with an average ratio of 1.56.

Although the average total cinnamoylcocaine content of South American ECVC coca leaf is in the range of 8–12%, the mean content in illicit refined cocaine samples is below 4% (relative to cocaine). There is little doubt that this marked decline in going from leaf to refined sample is due to the use of potassium permanganate in the clandestine cocaine manufacturing process. Potassium permanganate is used to oxidize the cinnamoylcocaines and thereby diminish their presence so as to allow for more effective crystallization in the cocaine base-to-cocaine hydrochloride conversion step.

9.1.2. Tropacocaine

As seen in Table 2, tropacocaine is present in Peruvian and Bolivian coca leaf at much lower levels than the cinnamoylcocaines and is therefore less readily detected in refined cocaine samples. Janzen *et al.* [29] used cGC–NPD to detect tropacocaine in each of a representative set of 10 cocaine samples. In a survey of 71 cocaine samples using packed column GC–FID, Ensing *et al.* [30] reported tropacocaine in about 75% of them. Neither of these two studies provided absolute quantitative data for tropacocaine. In the study by Casale and Waggoner [26,72] of 4000 cocaine samples, tropacocaine was detected and quantitated in over half of the exhibits examined, with levels varying from 0.01% to as high as 0.9% (relative to cocaine), with most samples rarely exceeding 0.1%. The mean tropacocaine content for the 4000 samples was found to be 0.02%.

From Table 2 and other data [72], there appears to be more than an order of magnitude decline in the tropacocaine content from the processing of leaf to refined cocaine. The reason for the marked decrease in this alkaloid is not clear, but it may occur during the potassium permanganate oxidation step, or more likely, due to its solubility characteristics.

9.1.3. Truxillines

Although the presence of α- and β-truxilline in coca leaf was first described by Hesse [73] and Liebermann [74] in the late 19th century, it was not until 1987 that Moore *et al.* [14] first reported them and nine related isomers in illicit refined cocaine samples (Fig. 4). The structural elucidation of the truxillines was accomplished by first removing them from the bulk cocaine matrix using alumina column chromatography. The combined truxillines were acid hydrolyzed to their corresponding truxillic/truxinic acids and then converted to their respective dimethyl esters. The mixture of dimethyl esters were then resolved by preparative HPLC [14] and characterized by cGC–MS and ^{1}H NMR. Because of their considerable mass ($M_r = 658$), the truxillines are difficult to chromatograph directly by GC, usually undergoing thermal degradation in the injection port to yield ecgonidine methyl ester and the truxillic/truxinic acids. In order to determine the truxillines by GC, they were first

reduced and then derivatized as previously described for coca leaf (Fig. 5). As in the coca leaf analysis, the truxillines were then determined in refined samples by cGC–ECD. In a follow-up investigation, Moore [18] determined the total truxilline content of about 130 refined cocaine samples by cGC–ECD and found that their levels varied from 0.2% to 12% (relative to cocaine). The accuracy and reproducibility of that methodology was enhanced by the incorporation of μ-truxinic acid as a structurally related internal standard (note: it had been previously determined by the author that the μ-truxilline content of illicit refined cocaine samples to be negligible, thus allowing the use of this isomer as an internal standard). Table 7 gives the total individual truxilline content for 10 representative illicit cocaine samples seized in South America.

Preliminary truxilline data from Peruvian and Bolivian coca leaf (Table 4) and recent truxilline data from the analysis of about 50 refined illicit cocaine samples from Peru, Bolivia and Brazil [71] suggested that, in the processing of coca leaf to refined cocaine, the truxillines experienced a much higher carry-through than either the cinnamoylcocaines or tropacocaine. It was emphasized by the authors [18,35,71], however, that the truxilline content of many more Peruvian and Bolivian coca leaf samples need to be determined to confirm those preliminary findings.

To circumvent the thermal degradation associated with the GC analysis of the truxillines, Lurie *et al.* [15,21] presented methodology involving HPLC with photodiode-array UV detection. In one of those methods [15] the truxillines were chromatographed intact after direct injection of the cocaine sample on an HS-5 C_{18} column followed by gradient elution with a solvent mixture of acetonitrile–phosphate buffer (0.02 *M* dodecyl sulfate, pH 2.0). Fig. 10 (top) illustrates this chromatography. It is noted that, in addition to the truxillines, the cinnamoylcocaines and several manufacturing by-products were also detected by this methodology. In Fig. 10 (bottom) the truxillines were isolated via size-exclusion chromatography [21]. Recently, Ensing and De Zeeuw [27] described the thin-layer chromatographic separation and mass spectral identification of five truxillines in illicit cocaine samples seized in the Netherlands Antilles.

Table 7
Individual and total truxilline content ("intact" truxillines + truxilline hydrolysis products) of ten representative refined illicit cocaine hydrochloride samples seized in South America (see ref. 18)

Truxilline	Sample numbers									
	1	2	3	4	5	6	7	8	9	10
ϵ	0.84	0.64	0.49	0.36	0.16	0.15	0.12	0.14	0.11	0.03
δ	1.06	0.81	0.72	0.48	0.15	0.22	0.18	0.23	0.17	0.06
β	2.11	1.48	1.60	0.94	0.60	0.55	0.54	0.42	0.41	0.04
peri + neo-	0.48	0.40	0.36	0.19	0.08	0.09	0.06	0.06	0.04	0.02
epi	0.05	0.08	0.05	0.02	0.01	0.02	0.01	0.01	0.01	0.00
α	3.13	2.68	1.80	1.00	0.75	0.51	0.31	0.38	0.30	0.06
ω	0.69	0.41	0.33	0.17	0.15	0.06	0.03	0.05	0.03	0.01
γ	0.50	0.42	0.28	0.14	0.12	0.05	0.03	0.04	0.02	0.01
μ	–	–	–	–	–	–	–	–	–	–
ζ	0.15	0.09	0.13	0.07	0.06	0.04	0.04	0.03	0.03	0.02
Total	9.01	7.01	5.76	3.37	2.08	1.69	1.32	1.36	1.12	0.25

A total of 130 illicit cocaine hydrochloride samples from South America were subjected to total truxillines quantitation, of which 10 sample results were selected at random. All results are % (w/w), relative to cocaine. An internal standard of μ-truxinic acid was used as a reference standard for all calculations, and similar molar ECD responses and concentration/response linearity is assumed for all truxillines.

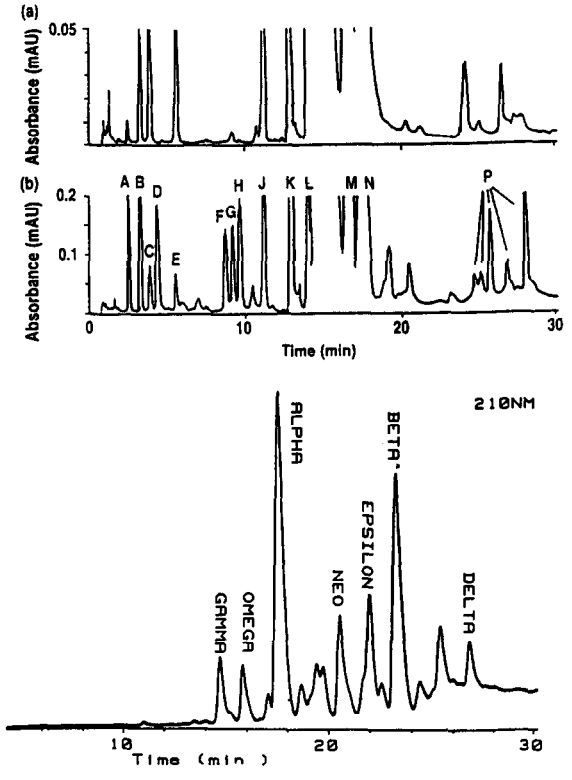


Fig. 10. (Top) HPLC chromatograms obtained at (a) 277 nm and (b) 215 nm from the separation of a cocaine hydrochloride sample. Peaks: A = meconin (internal standard); B = benzoic acid; C = *cis*-cinnamic acid; D = α-truxillic acid; E = *trans*-cinnamic acid; F = ϵ-truxillic acid; G = β-truxinic acid; H = δ-truxinic acid; J = *n*-butyrophenone (internal standard); K = benzoylecgonine; L = cocaine; M = *cis*-cinnamoylcocaine; N = *trans*-cinnamoylcocaine; P = truxillines. From ref. 47. (Bottom) HPLC of illicit cocaine impurities including isomeric truxillines isolated via size-exclusion chromatography (see ref. 21).

9.1.4. Hydroxycocaines

In a 1993 report, Moore and Cooper [33] described for the first time the detection of 4–8 suspected hydroxycocaines in refined cocaine samples. After their isolation from the bulk cocaine matrix using alumina column chromatography, the hydroxycocaines were presumptively identified by MS as HFB and trimethylsilyl derivatives. 6-Hydroxycocaine, seen in Fig. 8, is suspected of being one of the hydroxycocaines present in cocaine samples. Over 100 unadulterated and refined cocaine hydrochloride samples from South America were subjected to derivatization with HFBA, and the resultant HFB derivatives of the hydroxycocaines, and other derivatizable compounds, were subjected to cGC–ECD analyses. The hydroxycocaines were detected in all samples examined, with most at individual concentration levels below 0.01% (relative to cocaine). Partial chromatograms of the hydroxycocaines and other impurities/by-products present in two illicit cocaine samples are illustrated in Fig. 11 [33]. The chromatography

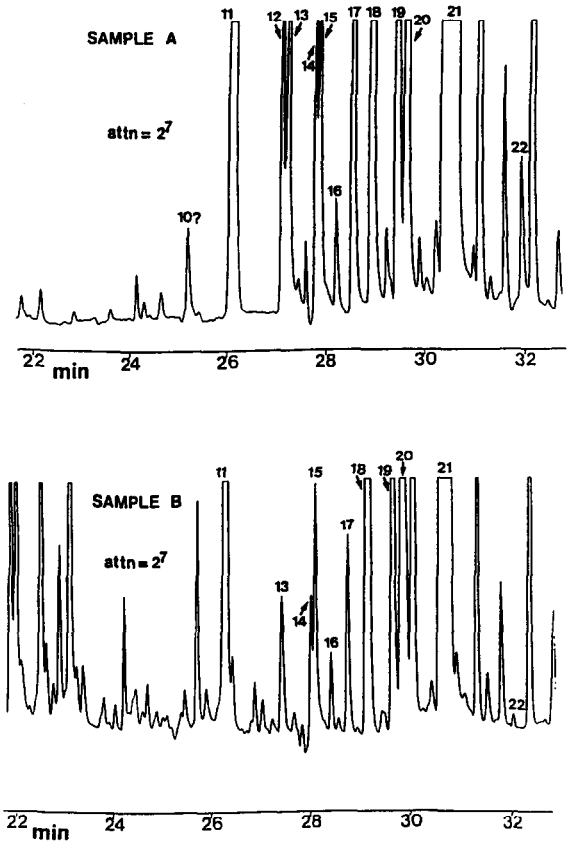


Fig. 11. Partial cGC–ECD chromatograms of heptafluorobutyryl derivatives of some cocaine manufacturing impurities/by-products in two unrelated and unadulterated illicit cocaine hydrochloride samples (30 m × 0.25 mm DB-1701). Peaks: 11 = heneicosanol internal standard (as O-HFB derivative); 12–18, 20 = suspected isomeric hydroxycocaines (as O-HFB derivatives; *e.g.*, see Fig. 8); 19 = N-benzoylnorecgonine methyl ester (as O-HFB derivative; see Fig. 13); 21 = N-norcocaine (as N-HFB derivative; see Fig. 1). From ref. 33.

was done on a 30 m × 0.25 mm fused-silica column coated with DB-1701.

9.1.5. 3′,4′,5′-Trimethoxy-substituted coca alkaloids

Using ion-pairing column chromatography, with 1 *M* HCl–2 *M* NaCl/Celite 545 as the stationary phase and water-saturated chloroform as the eluent, Casale and Moore [36] quantitatively isolated trace levels of the trimethoxy-substituted analogues of tropacocaine, cocaine and the cinnamoylcocaines, along with N-norcocaine and tropacocaine, from 15 South American coca paste and refined cocaine base and hydrochloride samples. These alkaloidal impurities were determined using cGC–FID and a 30-m DB-1 fused-silica capillary column. Of the eight refined cocaine base and hydrochloride samples, the levels of trimethoxycocaine were all below 0.05% (relative to cocaine), with most below 0.005%; trimethoxycocaine was not detected in two Peruvian cocaine hydrochloride samples. It was also interesting to note that of the 13 illicit refined and paste samples with measureable levels of the trimethoxy compounds, eight had *cis*- + *trans*-cinnamoylcocaine levels higher than trimethoxycocaine. This was also in agreement for three of the South American coca leaf samples, in which the total trimethoxycinnamoylcocaines/trimethoxycocaine ratio was about 5 (Table 5). However, this was in sharp contrast to the average cocaine/*cis*- + *trans*-cinnamoylcocaine ratio of 40 for illicit refined samples. Overall, there were sharp declines in the concentrations of trimethoxy-containing alkaloids in the processing of leaf to refined cocaine, in some cases more than an order-of-magnitude decrease. The cGC–FID chromatogram of trimethoxy-substituted alkaloidal and other known and unknown impurities in a refined South American cocaine base sample is illustrated in Fig. 12.

9.1.6. Cuscohygrine and hygrine

Although methodology has been recently developed for the detection of cuscohygrine and hygrine in refined cocaine samples [34], there have been no reports of their presence in that

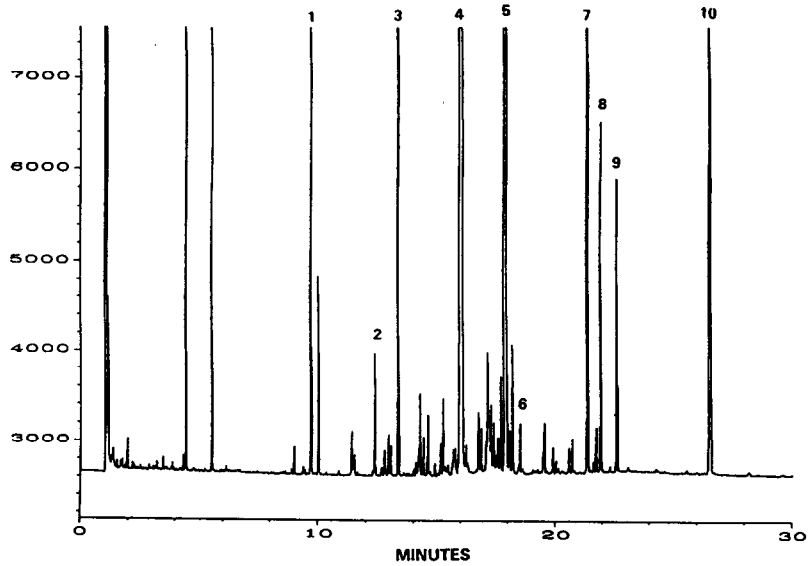


Fig. 12. The cGC–FID chromatogram (30 m × 0.25 mm I.D. DB-1) of ion-pair eluate from a refined cocaine base sample containing trimethoxy-substituted alkaloidal impurities and other compounds. See Figs. 1 and 9 for structures. Peaks: 1 = tropacocaine; 2 = N-norcocaine; 3 = cocaine; 4 = *cis*-cinnamoylcocaine; 5 = *trans*-cinnamoylcocaine; 6 = 3′,4′,5′-trimethoxy-tropacocaine; 7 = 3′,4′,5′-trimethoxycocaine; 8 = 3′,4′,5′-trimethoxycocaethylene (internal standard); 9 = 3′,4′,5′-trimethoxy-*cis*-cinnamoylcocaine; 10 = 3′,4′,5′-trimethoxy-*trans*-cinnamoylcocaine (see Fig. 9). From ref. 36.

matrix. It is suspected that their levels in refined cocaine would be very low because of their high solubility in aqueous solutions and higher volatility, resulting in substantial losses during the manufacturing process.

9.2. Manufacturing by-products in illicit refined cocaine

9.2.1. Alkaloid hydrolysis products

Varying amounts of manufacturing by-products are present in virtually all illicit refined cocaine samples. The most readily detected by GC–FID and/or HPLC–UV are cocaine hydrolysis products, *i.e.*, benzoylecgonine, ecgonine methyl ester, ecgonine and benzoic acid (Fig. 1) [2,3,6,8,10,15,20,26]. Additional hydrolysis by-products include the cinnamic acids (Fig. 1) [26], which are derived from the cinnamoylcocaines, and a multitude of other trace level products from the hydrolysis of the isomeric truxillines, including truxillic and truxinic acids (Fig. 4) [14,26]. Most of the aforementioned hydrolytes are probably produced during the manufacturing process; however, some, especially those derived from the truxillines, can be formed during prolonged storage [18].

9.2.2. Cocaine oxidation by-products

Other cocaine manufacturing by-products detected by GC–FID and/or cGC–ECD at trace levels in refined samples include N-norcocaine (Fig. 1) [17,26,28,29,33], N-formylnorcocaine (Fig. 13) [24,28,30], N-benzoylnorecgonine methyl ester (Fig. 13) [28,30,33] and N-norecgonine methyl ester [33]. Ensing and Hummelen [28] have attributed the first three of these by-products to the use of potassium permanganate in the cocaine manufacturing process. These authors also described the relationship between N-norcocaine and N-benzoylnorecgonine methyl ester as being pH dependent. It is also believed that N-norcocaine can be formed by the peroxide-assisted N-demethylation of cocaine [33]. Peroxides are often present in certain solvents, notably diethyl ether, used in the manufacture of cocaine and other drugs. N-Norecgonine methyl ester, which has been detected by cGC–ECD [33], is probably the result of N-norcocaine hydrolysis.

The quantitative levels of the above by-products can vary by several orders of magnitude in illicit refined cocaine samples. Cocaine hydrolysis products are often present in samples at levels over 1% and are readily detected by either cGC–FID or HPLC–UV. The other manufacturing by-products are usually detected by cGC–FID, and especially by cGC–ECD, at levels well below 1%.

9.2.3. Solvent residues

The analysis of solvents as contaminants/by-products in illicit refined cocaine samples has been only recently reported. Methodology that has been applied in the detection of solvent residues have utilized ^{1}H NMR spectroscopy and headspace GC. In 1991, Avdovich *et al.* [41] described the ^{1}H NMR analysis of over 150 illicit refined cocaine exhibits and reported the presence of seven solvents in those samples. By far the two most commonly seen solvent residues were acetone and methyl ethyl ketone, followed by diethyl ether, benzene and toluene. The minimum detectability of selected solvents, based upon a 200-mg sample mass, was benzene (100 ppm), toluene (150 ppm), acetone (100 ppm), diethyl ether (250 ppm) and methyl ethyl ketone (200 ppm). Quantitative estimations of

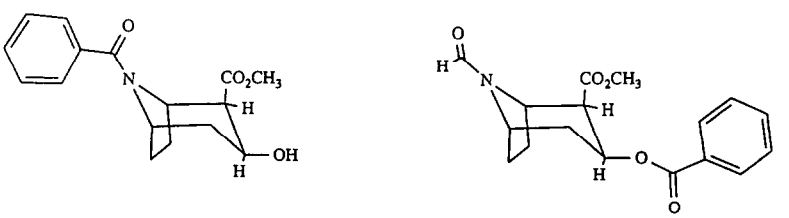


Fig. 13. Structures for N-benzoylnorecgonine methyl ester (left) and N-formylnorcocaine (see refs. 28 and 33).

solvents in 36 samples revealed ppm residue ranges from 400–11000 (acetone), 1600–7700 (methyl ethyl ketone), 700–1200 (diethyl ether), 100–600 (benzene) and 800–2300 (toluene).

Kram [42] also utilized ^{1}H NMR for solvent analyses and reported for the years 1986–1990 similar solvents as described by Avdovich *et al.* [41]. However, Kram [42] listed somewhat lower minimum detectability levels of 10–70 ppm for the most common solvent residues. It is interesting to note in the Kram study, that from 1985 to 1990 the detection of acetone and diethyl ether in cocaine samples declined markedly, while solvents such as methyl ethyl ketone, aliphatic hydrocarbons, toluene, methyl chloride and ethyl acetate were seen with much greater frequency.

In the most comprehensive solvent residue study done to date, Fortuna [43] compiled the headspace GC qualitative results of 1702 cocaine samples received from forensic laboratories during the period of 1986–1991. The data revealed that methyl ethyl ketone was the most frequently detected residue (74.1% of samples), followed by toluene (57.4%), methylene chloride (49.1%), ethyl acetate (36.6%), aliphatic hydrocarbons (33.3%), acetone (25.8%), benzene (18.8%), methyl acetate (14.1%) and diethyl ether (3.9%). Other solvent residues were also present, but were encountered with less frequency.

In what is perhaps the most sensitive solvent residue method to date, Morello and Myers [44] reported quantitative data for solvents in illicit refined cocaine samples using headspace GC–MS with ion-trap detection. Twenty-five of the most commonly encountered solvents provided linear responses over significantly different ranges, with minimum detection limits ranging from 0.1 to 5.7 μg (present in headspace chamber), depending upon the solvent. Minimum detection limits (based upon 45 mg sample mass) for several commonly encountered solvents occluded in cocaine hydrochloride samples included hexane 0.5 ppm, methylene chloride 1 ppm, toluene 1 ppm, diethyl ether 3 ppm, methyl ethyl ketone 13 ppm, ethyl acetate 14 ppm and acetone 48 ppm. The use of deuterated solvents as internal standards in this methodology enhanced its quantitative accuracy and reproducibility. Recently, this method was used successfully in a cocaine sample comparison case.

9.3. Electron ionization mass spectra of coca alkaloids and by-products present in coca leaf and/or refined illicit cocaine

Found in the Appendix are the electron ionization mass spectra (mass-selective detection) of old and recently-reported alkaloids, as well as manufacturing by-products reported to be present in ECVC coca leaves and/or illicit refined cocaine samples. All mass spectra were generated at the US Drug Enforcement Administration's Special Testing and Research Laboratory.

10. Cocaine impurity signature profiles

Since about 1988 there has been a spate of chromatographic methods reported for the simultaneous detection and determination of alkaloidal impurities and manufacturing by-products in illicit refined cocaine samples. These so-called "impurity signature profiles" were usually developed for the comparative analyses of cocaine seizures. This was accomplished primarily by packed-column or cGC–FID, cGC–NPD, cGC–ECD, cGC–MS and HPLC–UV.

10.1. Methods 1–4

The earliest reports of a cocaine impurity signature profile were by Moore in 1974 [2] and again in 1978 [3]. In those procedures, unadulterated cocaine samples were subjected to derivatization with N,O-bis-(trimethylsilyl)-acetamide (BSA) followed by packed-column GC–FID analysis. The impurities and by-products detected included the cocaine hydrolysis products (Fig. 1), all chromatographed as trimethylsilyl (TMS) derivatives, and the cinnamoylcocaines. Soon after, Clark [4] described the first packed-column GC-FID quantitation of the cinnamoylcocaines, ecgonine methyl ester and ecgonine in a cocaine impurity signature

profile. The cinnamoylcocaines were hydrolyzed to their respective cinnamic acids, which were then determined as methyl esters, while in a separate analysis, ecgonine methyl ester and ecgonine were quantitated as TMS derivatives. In a subsequent study, done without chemical derivatization, Lukaszewski and Jeffery [6] chromatographed some of these same compounds on a packed column with MS detection. The authors identified the presence of methylecgonidine and ethylecgonine in the chromatographic profiles as artifacts, created in the injection port of the GC.

10.2. Method 5

The isomeric truxillines have also been used successfully in impurity signature profiles of refined cocaine samples [18,75]. This group of compounds is attractive for sample comparison analyses because of the great number of absolute and relative mathematical permutations of the generated data that are possible. This is because the total truxilline content of refined illicit cocaine samples can vary from 1–2 orders of magnitude [71]. Furthermore, there are 10 easily detected individual truxilline isomers that can provide numerous intra-truxilline ratios. The use of μ-truxinic acid as a structurally related internal standard allows for enhanced reproducibility and accuracy [18]. Recently, the truxilline methodology was instrumental, in part, for a successful federal criminal prosecution of a cocaine comparison case [75].

10.3. Method 6

In the most comprehensive study done to date, Casale and Waggoner [26] markedly improved upon Moore's methodology [3] by using a capillary column, in lieu of a packed column, for the development of a cocaine impurity signature profile. After BSA derivatization of the cocaine sample, it was chromatographed on a 30-m fused-silica capillary coated with DB-1701 (0.26 μm) with detection by FID. The capillary column allowed for improved resolution and increased sensitivity and resulted in the detection of many more compounds, including all those seen in Fig. 1 (excepting *p*-fluorococaine, which was used as an internal standard). Two cGC–FID chromatograms illustrating the chromatography of an illicit refined cocaine hydrochloride and cocaine base samples are presented in Fig. 14. The reproducibility and quantitative accuracy of the profiles were enhanced by the incorporation of a structurally related internal standard, *p*-fluorococaine (Fig. 1). Over 4000 samples were analyzed using this methodology and the data compiled in a computerized data base [26,72,76]. A detailed discussion of statistical analyses supporting the impurity chromatographic profiles, including principal component analyses, were provided by the authors. The ultimate goal was to build a computerized data base of samples to be searched against new exhibits for possible "matches". The authors reported that a new neural network pattern recognition analysis appeared promising for the establishment of a common source identity for each batch of cocaine [76]. This methodology, and a subsequent modification, have both been recently accepted by two federal courts in the successful criminal prosecution of a cocaine comparison case involving cocaine traffickers [75].

10.4. Methods 7 and 8

In another cocaine impurity profile study, LeBelle *et al.* [20] utilized HPLC–UV and cGC–MS (ion-trap detection) for the detection and quantitation of components in illicit cocaine exhibits. Using a dual-wavelength detector (220 and 280 nm), the HPLC on-column minimum detectable levels for benzoylecgonine and *trans*-cinnamoylcocaine were 10 and 15 ng, respectively. The authors described the utility of their methodology for the examination and comparison of cocaine exhibits. In a follow-up study, LeBelle *et al.* [25] used HPLC–UV to determine the alkaloidal ratios of cocaine and *cis*- and *trans*-cinnamoylcocaine for sample comparison purposes. In the same study, cocaine samples were subjected to an accelerated stability study to determine if the ratios of the three target alkaloids changed markedly. The samples were stored for two weeks under controlled humidity

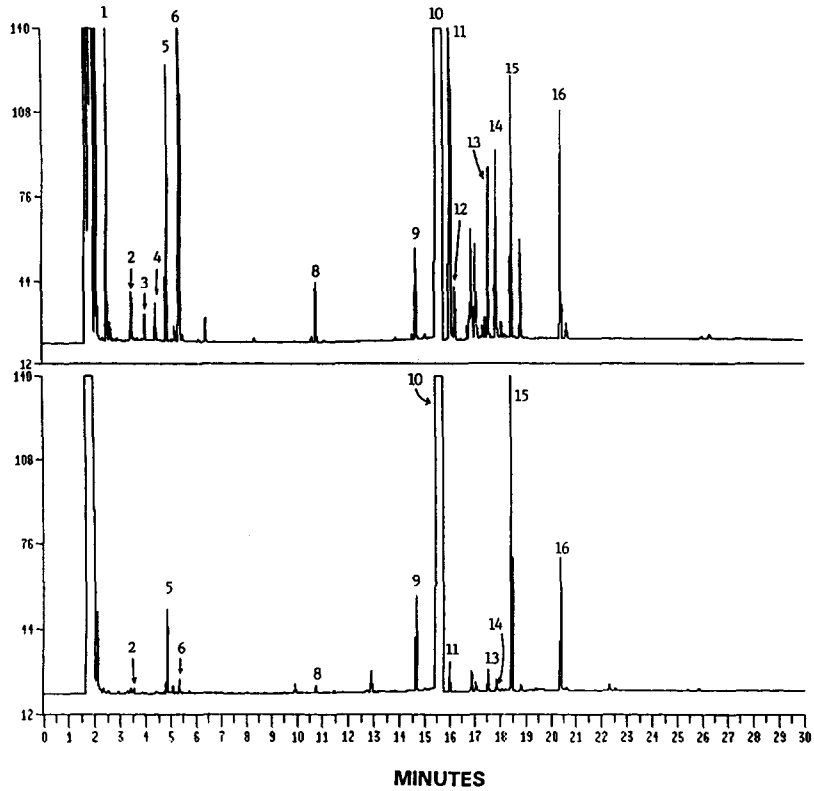


Fig. 14. cGC-FID (30 m × 0.25 mm I.D. DB-1701) of alkaloidal impurities and manufacturing by-products in (top) illicit refined cocaine hydrochloride sample and (bottom) illicit refined cocaine base sample. See Fig. 1 for peak identification and structures. From ref. 26.

of 50 and 75% and at a temperature of 60°C. No significant variance in ratios was noted. For more reliable sample comparisons, the alkaloidal ratios were complemented by the cGC–MS and cGC–FID analysis of acidic and neutral manufacturing by-products in cocaine samples. These by-products were isolated from the bulk cocaine matrix by extraction from a dilute acid solution into toluene. The cGC–MS analyses of these compounds revealed that some of them exhibited cocaine-like fragment ions; however, no structures were proposed.

10.5. Method 9

In an investigation using cGC–NPD, Janzen *et al.* [29] developed cocaine impurity signature profiles by computing the ratios of the peak area for cocaine to the peak areas for tropacocaine, N-norcocaine and *cis*- and *trans*-cinnamoylcocaine. Stability studies conducted under a variety of conditions suggested, and in agreement with LeBelle *et al.* [25], that the area ratios of cocaine to the four target alkaloids were stable. Furthermore, the ratios of the target alkaloids to cocaine were reproducible for same-batch samples and varied widely in unrelated samples. This is a desirable condition when developing methodology for impurity signature profiles. Also examined was the intra-sample variability of kilogram batches of cocaine; it was concluded such variations were small when compared with the variability in the general population. For the evaluation of data, a computer program was written to evaluate the Euclidian distances between a test sample and those contained in a library and to

locate those library samples that were closest to the test sample.

10.6. Method 10

Ensing *et al.* [30] applied packed-column GC–FID in the "fingerprinting" of illicit cocaine samples seized in the Netherlands Antilles. The impurity profiles were based on the presence or absence of six congeners, namely, tropacocaine, N-norcocaine, N-benzoylnorecgonine methyl ester, N-formylnorcocaine, and *cis*- and *trans*-cinnamoylcocaine. This method utilized the relative ratios of these compounds in the analyses of over 70 unrelated cocaine samples. The data revealed that a great variation was observed in the parameter composition and could be expressed numerically or graphically in the form of pictograms for easy visual comparison.

10.7. Method 11

In the most recent report of a cocaine impurity signature profile, Moore and Cooper [33] described the chemical derivatization of unadulterated cocaine samples with HFBA followed by cGC–ECD analyses. This methodology, referred to as the N-norcocaine method because of the usual dominance of the N-norcocaine chromatographic peak, is by far the most sensitive to date for the chromatographic detection of selected alkaloidal impurities as well as manufacturing by-products. In the analysis of over 100 illicit refined cocaine samples, N-norcocaine was easily detected in all samples, always as an off-scale peak at nominal sensitivity. Likewise, the hydroxycocaines, which were presumptively identified by MS, were detected in all samples, but at significantly lower levels than for N-norcocaine. The hydroxycocaines (*e.g.* Fig. 8) were believed to represent the most significant impurities in terms of sample discrimination. Unlike the truxillines, in which 10 of the 11 isomers were detected in all cocaine samples and had somewhat similar inter-isomer ratios, some of the hydroxycocaine isomers were present in some samples and not in others and varied more widely in inter-isomer ratios. Among other compounds that could be detected by this method were N-benzoylnorecgonine methyl ester, ecgonine methyl ester, N-norecgonine methyl ester, ψ-tropine, N-nor-ψ-tropine and other hydroxy-containing tertiary amine impurities and other N-nor compounds. Two partial cGC–ECD chromatograms of unrelated cocaine samples, illustrating the most discriminatory retention time windows, are presented in Fig. 11. As seen in Fig. 15, this method demonstrated excellent reproducibility. This methodology also played a prominent role in a federal prosecution of a cocaine comparison case [75].

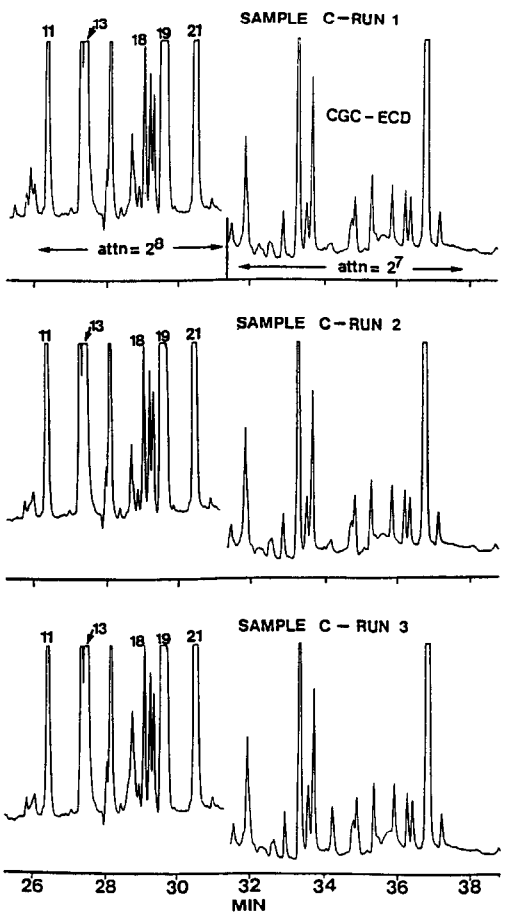


Fig. 15. The repetitive heptafluorobutyric anhydride derivatization and cGC-ECD analysis of an illicit refined cocaine hydrochloride sample (30 m × 0.25 mm I.D. DB-1701). For peak identification see Fig. 11. From ref. 33.

11. Signature profiles of adulterated and diluted cocaine samples

Virtually all of the foregoing profiling methods were applied to unadulterated and undiluted cocaine samples. However, the detection and quantitation of adulterants/diluents in illicit drugs can also be useful in sample comparison analyses and, in some instances, geographic origin determinations. For example, the presence of citric acid in two recent illicit cocaine seizures was so unusual that it proved to be a significant factor in a cocaine comparison case [75]. The most common adulterants and diluents of illicit cocaine include procaine, benzocaine, lidocaine, caffeine, boric acid, talc and sugars, such as inositol, mannitol, dextrose and lactose. Although a comparison of cocaine samples can be achieved by reporting only the qualitative presence of adulterants/diluents, these analyses are markedly enhanced by including quantitative data.

The development of chromatographic signature profiles using cocaine manufacturing impurities/by-products for adulterated and diluted cocaine samples can be problematic, particularly if chemical derivatization is used. That this is so is because cocaine samples are frequently "cut" with substances that possess labile protons, thus rendering them susceptible to derivatization. This can result in the appearance of spurious and interfering peaks in the chromatographic profile and/or total quenching of the derivatization reagent. Even adulterants/diluents that do not derivatize may interfere with pertinent chromatographic peaks of cocaine manufacturing impurities/by-products. Caution should also be excercized when attempting to isolate cocaine manufacturing impurities/by-products from sample adulterants and diluents, especially when using liquid–liquid extraction techniques; such treatment may create artifacts that subsequently appear in the chromatographic profile. Thus, some manipulations may cause unwanted hydrolysis of target compounds. Other impurities/by-products may exhibit anomalous solubility characteristics, thus discriminating against the efficient recovery for some of them. In other instances impurities in solvents, such as peroxides, may result in the N-demethylation of some amine compounds.

12. Internal standards, accuracy and reproducibility

In the development of impurity signature profiles using chromatographic methodology, the judicious selection of an internal standard can enhance accuracy and reproducibility of the data, especially if absolute quantitative data is desired. This is of particular importance in sample comparison cases that may result in court testimony by the chemist. Probably the most ideal internal standards for both accuracy and reproducibility are the isotopic analogues of the impurities under study for use with MS quantitation. Unfortunately, this requires a level of sophistication most forensic drug laboratories do not possess. In any case, other types of internal standards are available for use in impurity signature profiles. Although straight-chain hydrocarbons have been reportedly used, they are inferior to drug compounds that are structurally related to the manufacturing impurities under study. A close structural relationship can include compounds such as homologues, positional and geometric isomers. These kinds of internal standards are especially warranted for derivatization methodology or procedures involving liquid–liquid partition chromatography. In this review, four methods that incorporated structurally related internal standards were discussed; these included μ-truxinic acid [18], p-fluorococaine [26], cocaethylene [35] and 3′,4′,5′-trimethoxycocaethylene [36]. The use of structurally related internal standards usually results in improved method reproducibility, as evidenced by typical relative standard deviation values of ± 2–5% for most impurities and by-products. Structurally related internal standards are also strongly recommended when optimum quantitative accuracy is required.

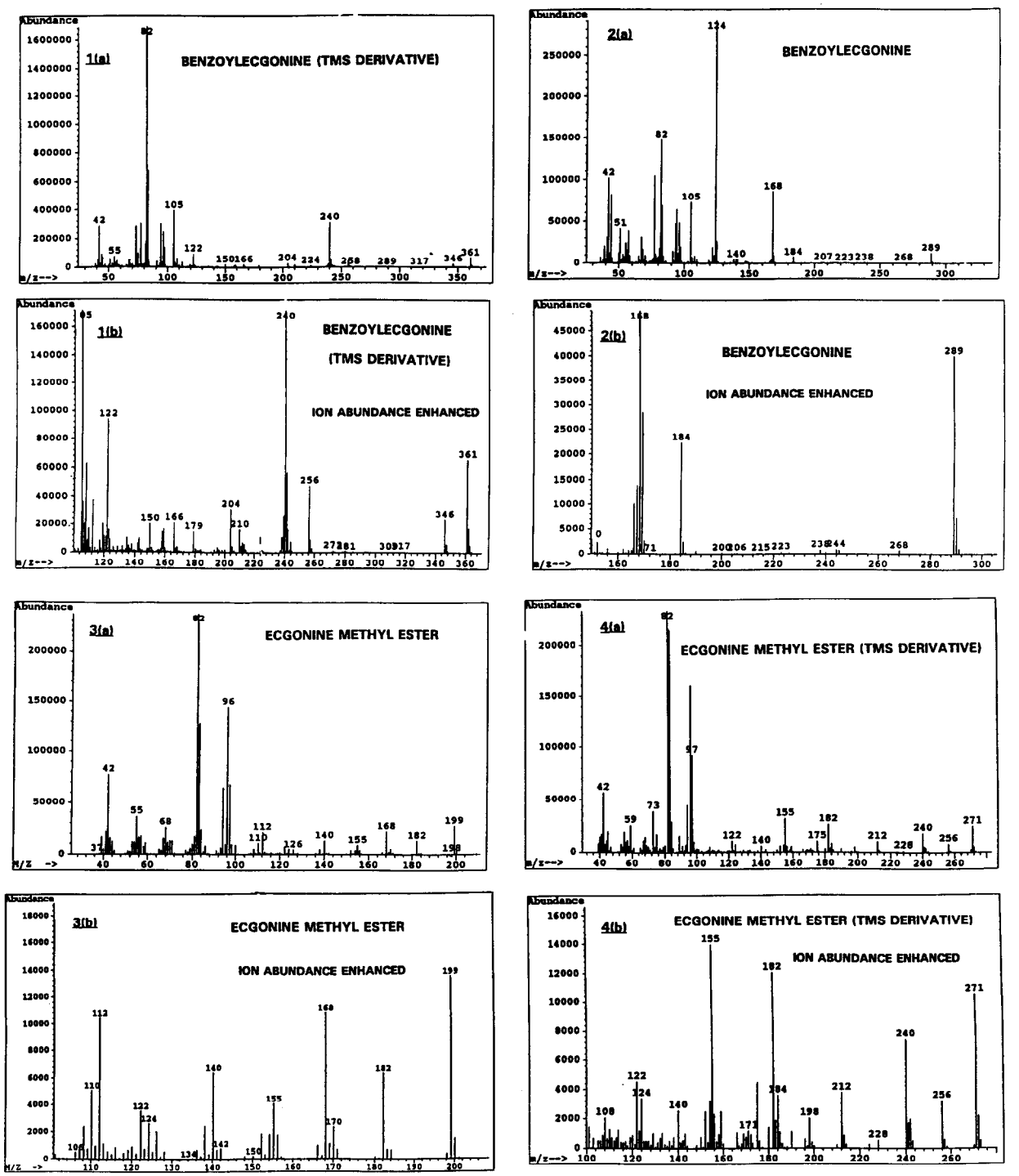


Fig. 16. Electron ionization mass spectra of alkaloids and manufacturing by-products detected in the leaves of South American *E. coca* var. *coca* and/or illicit refined cocaine samples.

(Continued on p. 194)

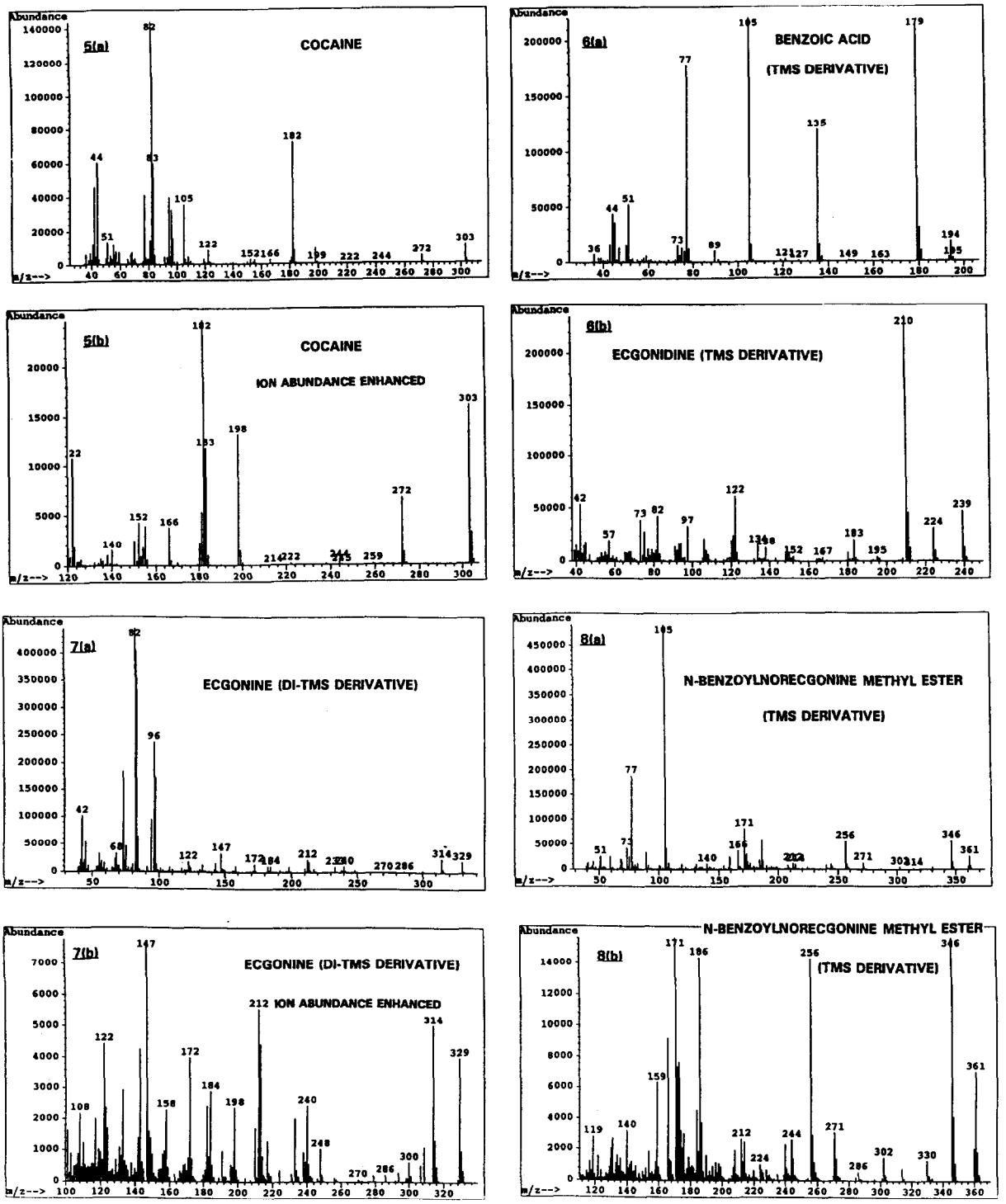

5(a)
COCAINE
6(a)
BENZOIC ACID
(TMS DERIVATIVE)
5(b)
COCAINE
ION ABUNDANCE ENHANCED
6(b)
ECGONIDINE (TMS DERIVATIVE)
7(a)
ECGONINE (DI-TMS DERIVATIVE)
8(a)
N-BENZOYLNORECGONINE METHYL ESTER
(TMS DERIVATIVE)
7(b)
ECGONINE (DI-TMS DERIVATIVE)
212 ION ABUNDANCE ENHANCED
8(b)
N-BENZOYLNORECGONINE METHYL ESTER
(TMS DERIVATIVE)
Abundance
m/z-->

Fig. 16.

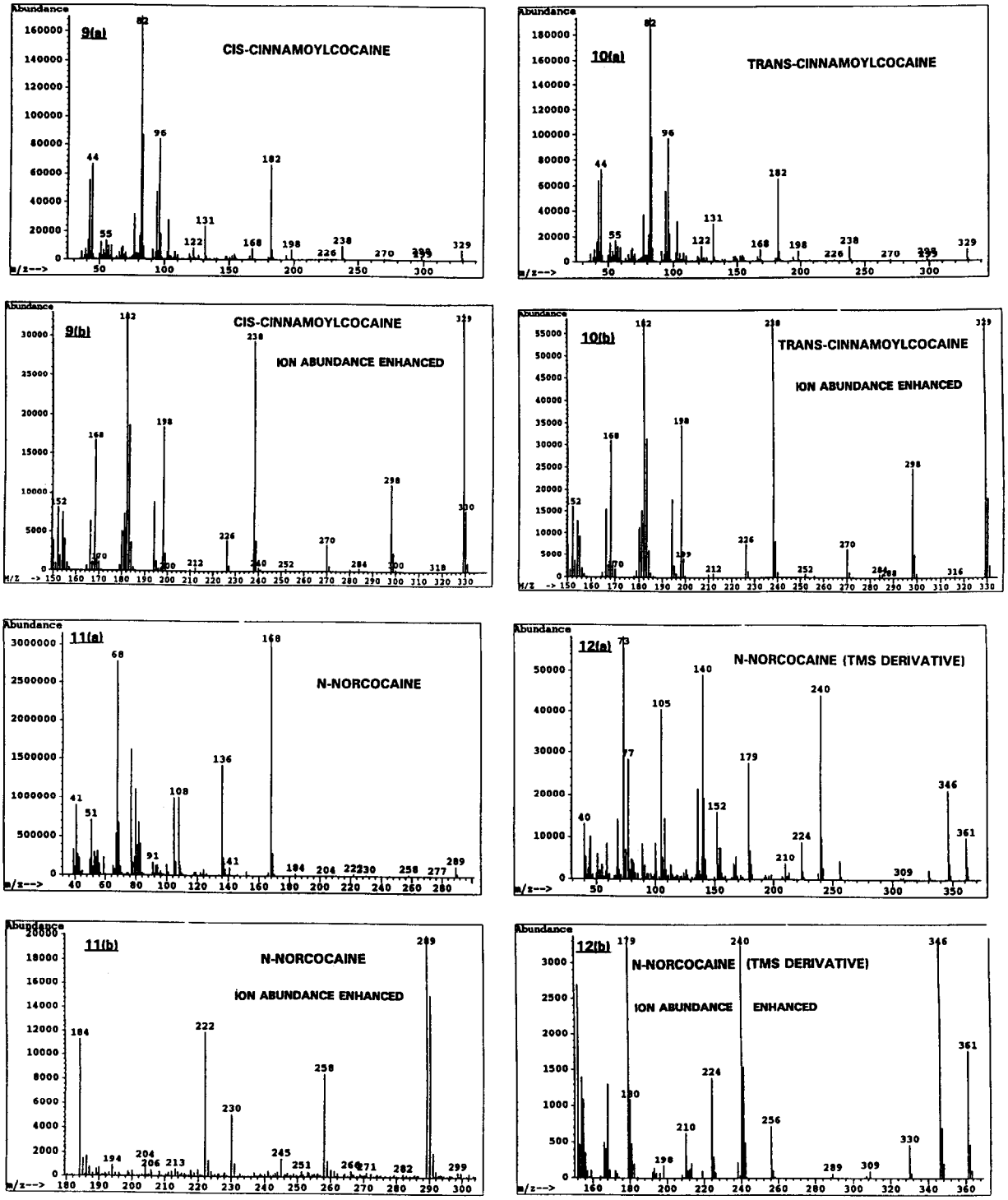


Fig. 16. *(Continued on p. 196)*

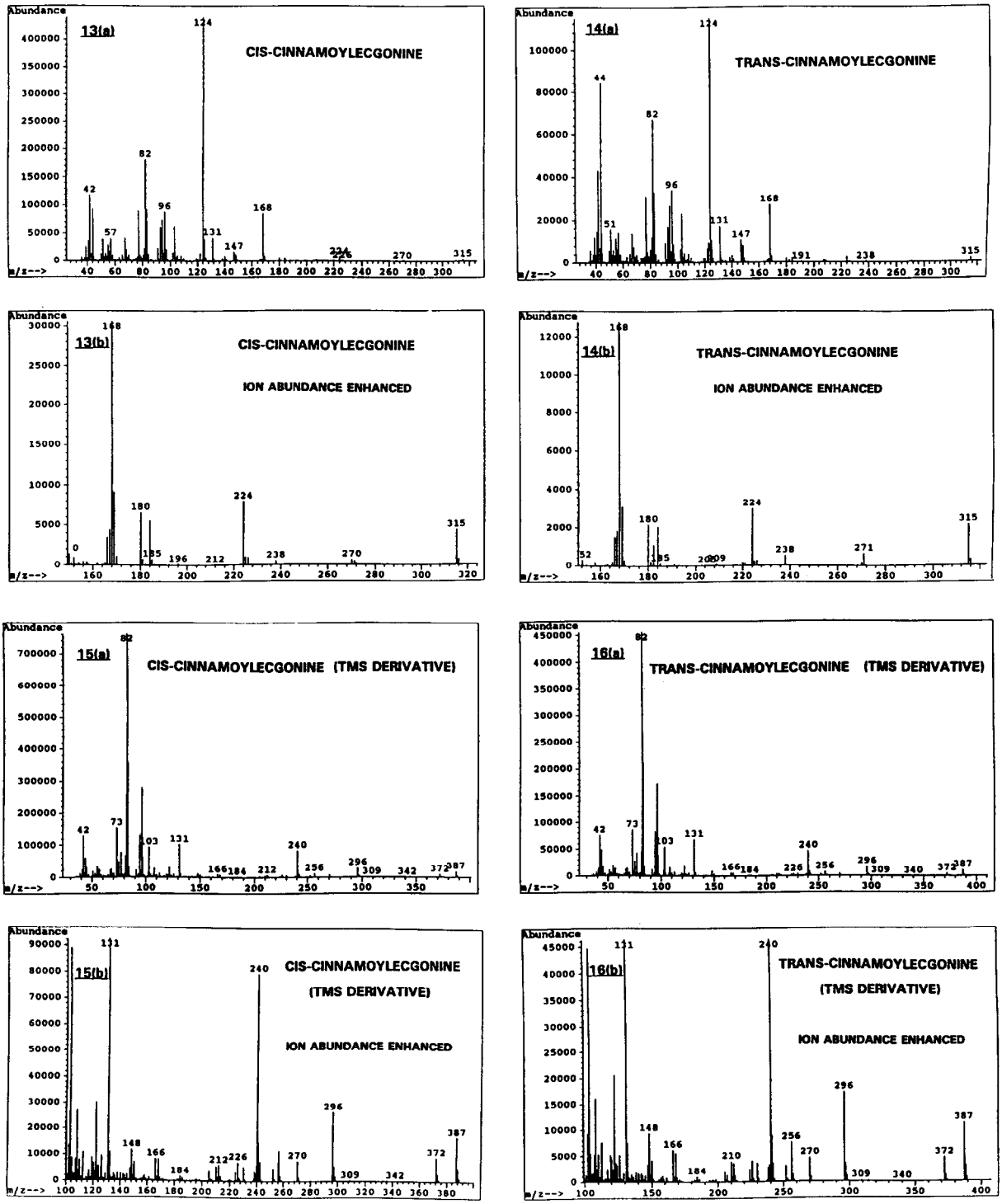

13(a)
CIS-CINNAMOYLECGONINE
14(a)
TRANS-CINNAMOYLECGONINE
13(b)
CIS-CINNAMOYLECGONINE
ION ABUNDANCE ENHANCED
14(b)
TRANS-CINNAMOYLECGONINE
ION ABUNDANCE ENHANCED
15(a)
CIS-CINNAMOYLECGONINE (TMS DERIVATIVE)
16(a)
TRANS-CINNAMOYLECGONINE (TMS DERIVATIVE)
15(b)
CIS-CINNAMOYLECGONINE
(TMS DERIVATIVE)
ION ABUNDANCE ENHANCED
16(b)
TRANS-CINNAMOYLECGONINE
(TMS DERIVATIVE)
ION ABUNDANCE ENHANCED
Abundance
m/z-->

Fig. 16.

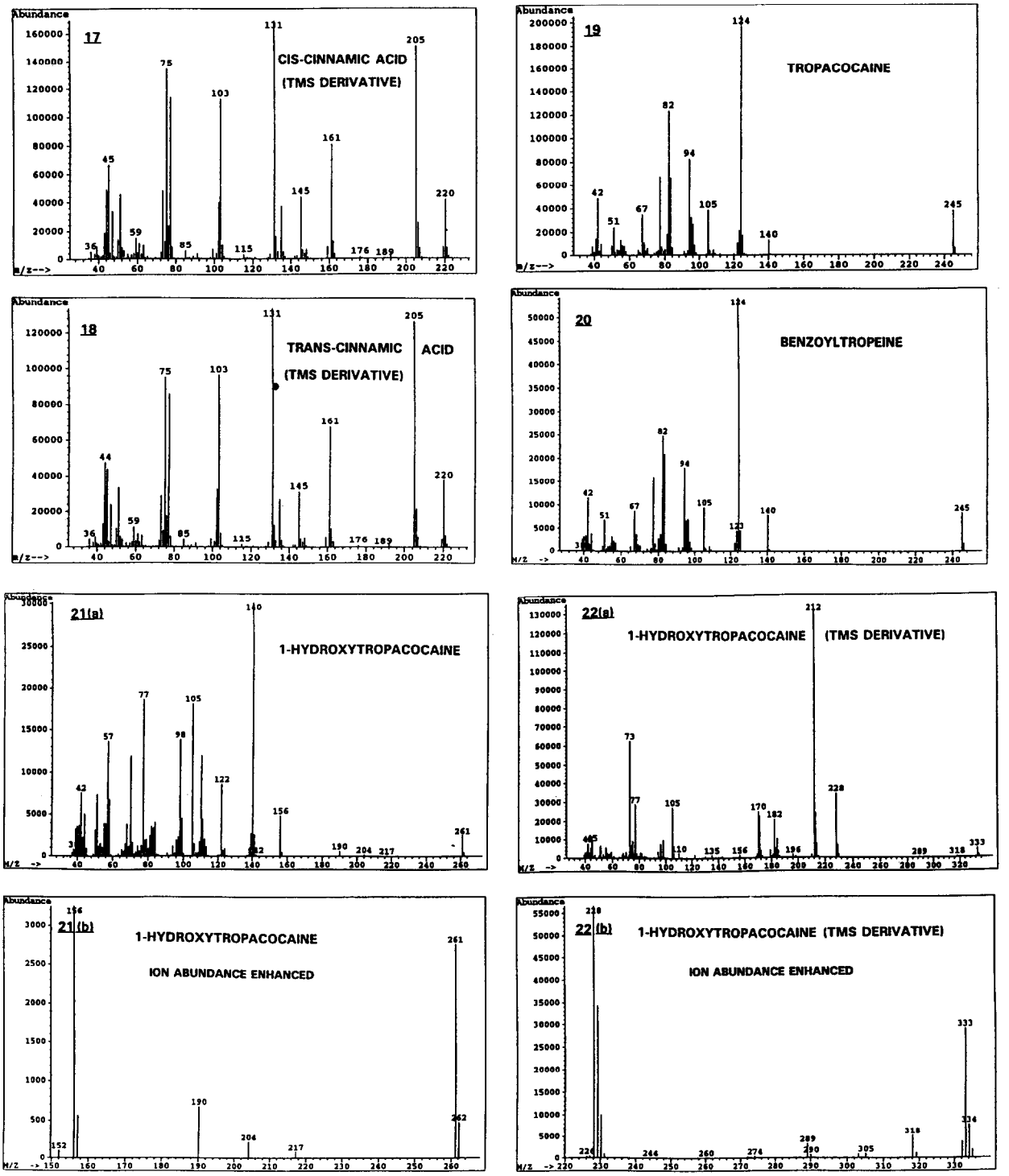
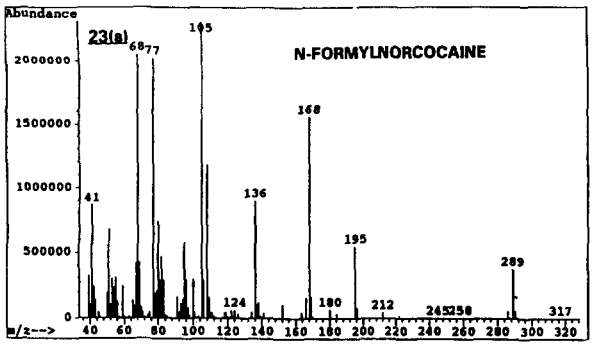
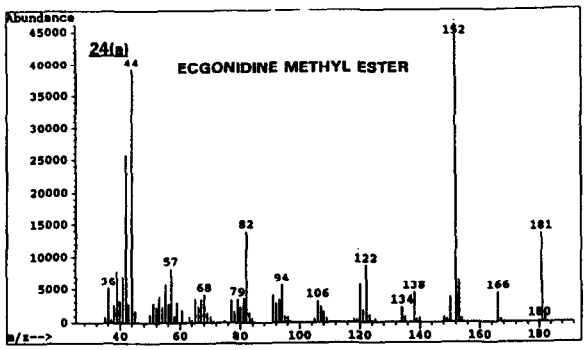
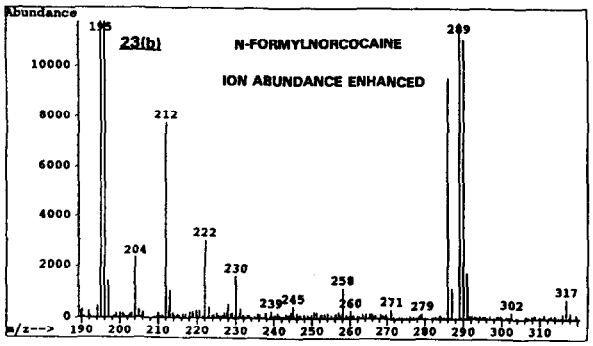
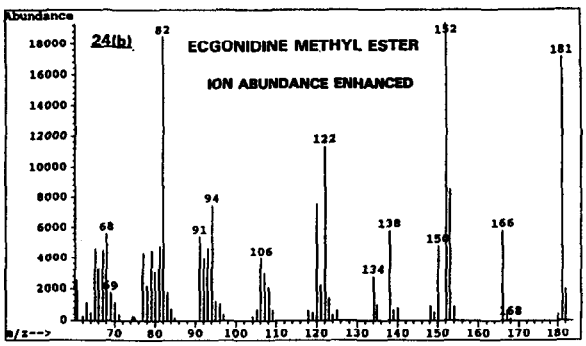


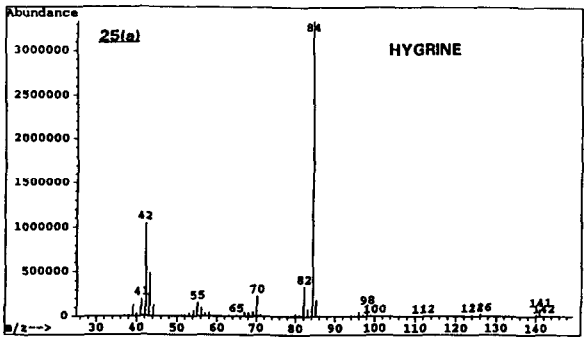
Fig. 16. *(Continued on p. 198)*

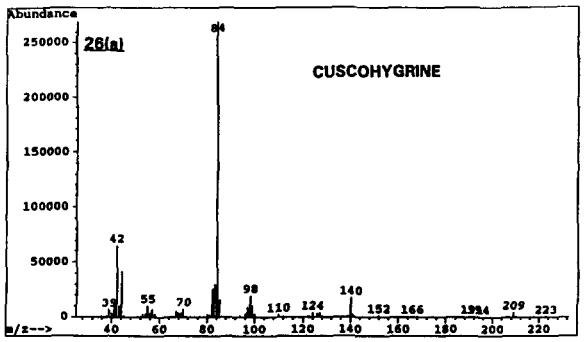

23(a)
N-FORMYLNORCOCAINE
Abundance
m/z-->

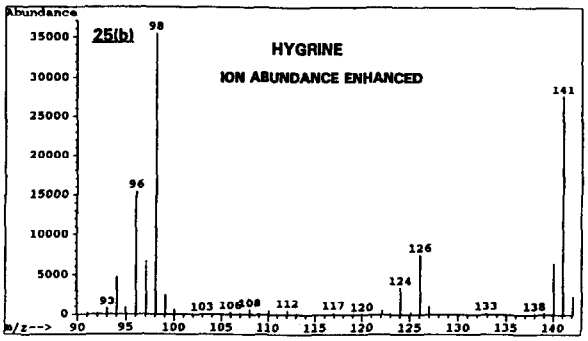

24(a)
ECGONIDINE METHYL ESTER
Abundance
m/z-->


23(b)
N-FORMYLNORCOCAINE
ION ABUNDANCE ENHANCED
Abundance
m/z-->


24(b)
ECGONIDINE METHYL ESTER
ION ABUNDANCE ENHANCED
Abundance
m/z-->


25(a)
HYGRINE
Abundance
m/z-->


26(a)
CUSCOHYGRINE
Abundance
m/z-->


25(b)
HYGRINE
ION ABUNDANCE ENHANCED
Abundance
m/z-->

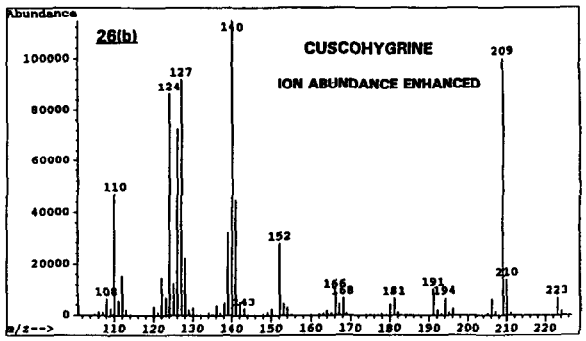

26(b)
CUSCOHYGRINE
ION ABUNDANCE ENHANCED
Abundance
m/z-->

Fig. 16.

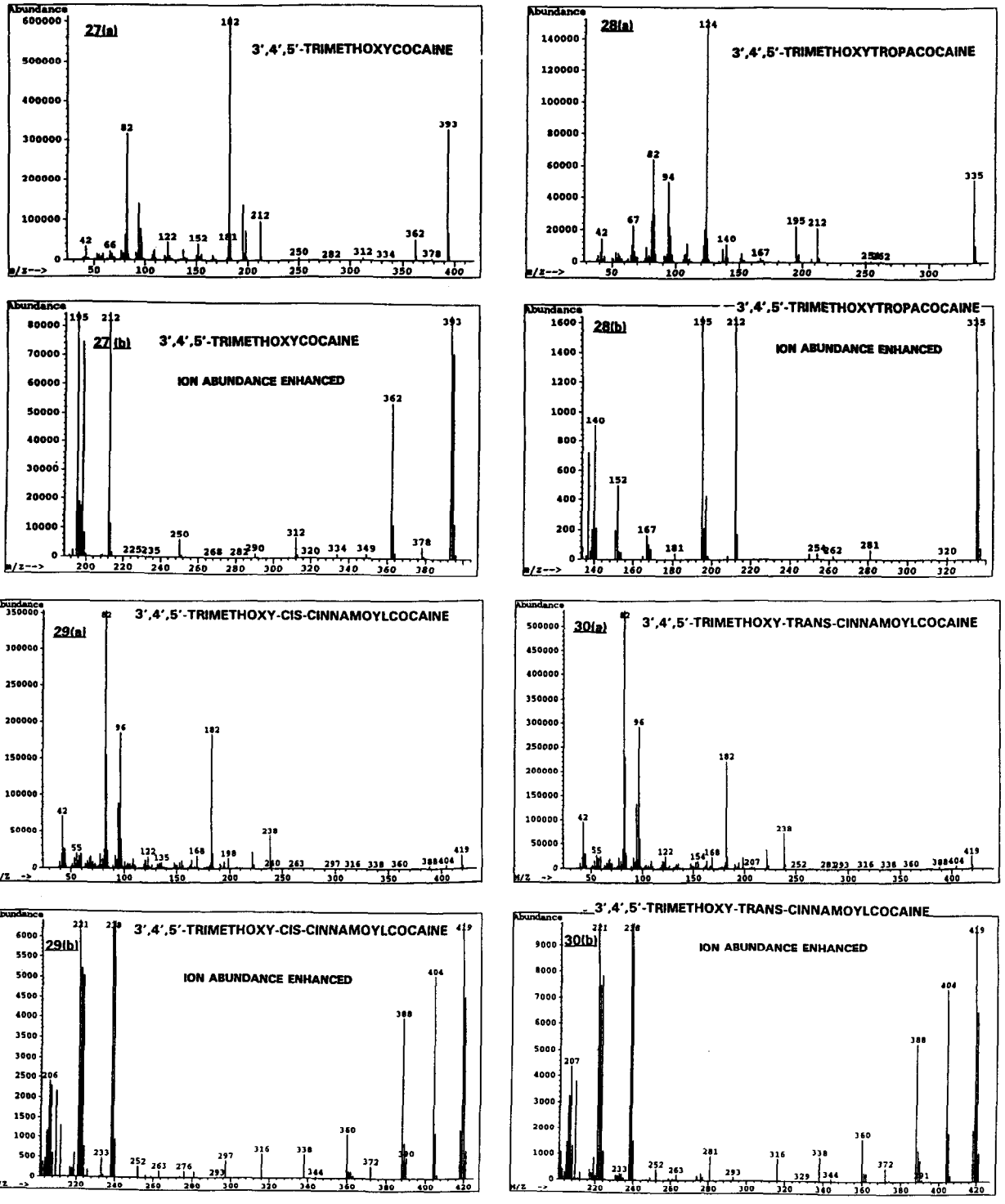


Fig. 16. *(Continued on p. 200)*

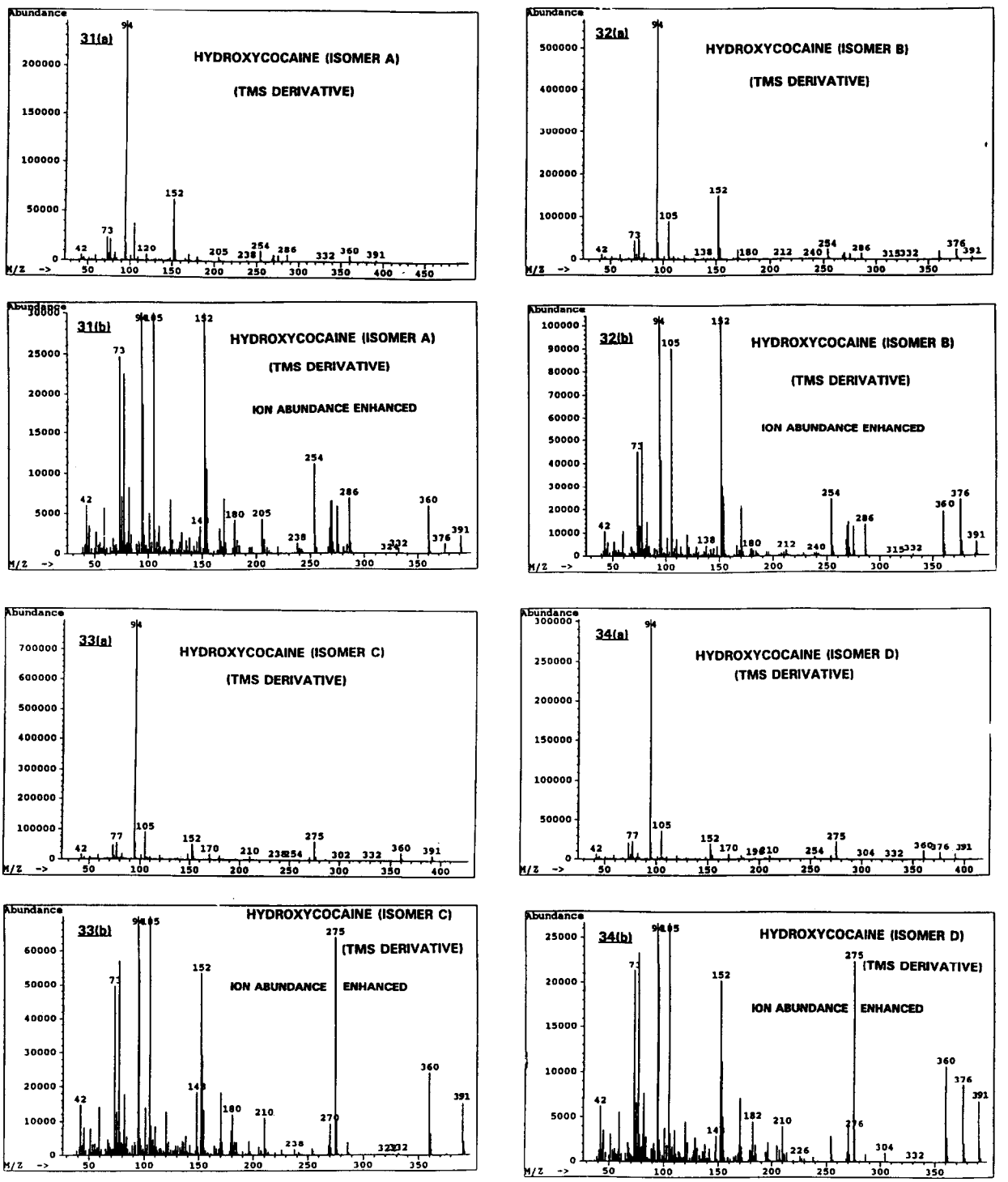

31(a)
HYDROXYCOCAINE (ISOMER A)
(TMS DERIVATIVE)
32(a)
HYDROXYCOCAINE (ISOMER B)
(TMS DERIVATIVE)
31(b)
HYDROXYCOCAINE (ISOMER A)
(TMS DERIVATIVE)
ION ABUNDANCE ENHANCED
32(b)
HYDROXYCOCAINE (ISOMER B)
(TMS DERIVATIVE)
ION ABUNDANCE ENHANCED
33(a)
HYDROXYCOCAINE (ISOMER C)
(TMS DERIVATIVE)
34(a)
HYDROXYCOCAINE (ISOMER D)
(TMS DERIVATIVE)
33(b)
HYDROXYCOCAINE (ISOMER C)
(TMS DERIVATIVE)
ION ABUNDANCE ENHANCED
34(b)
HYDROXYCOCAINE (ISOMER D)
(TMS DERIVATIVE)
ION ABUNDANCE ENHANCED

Fig. 16.

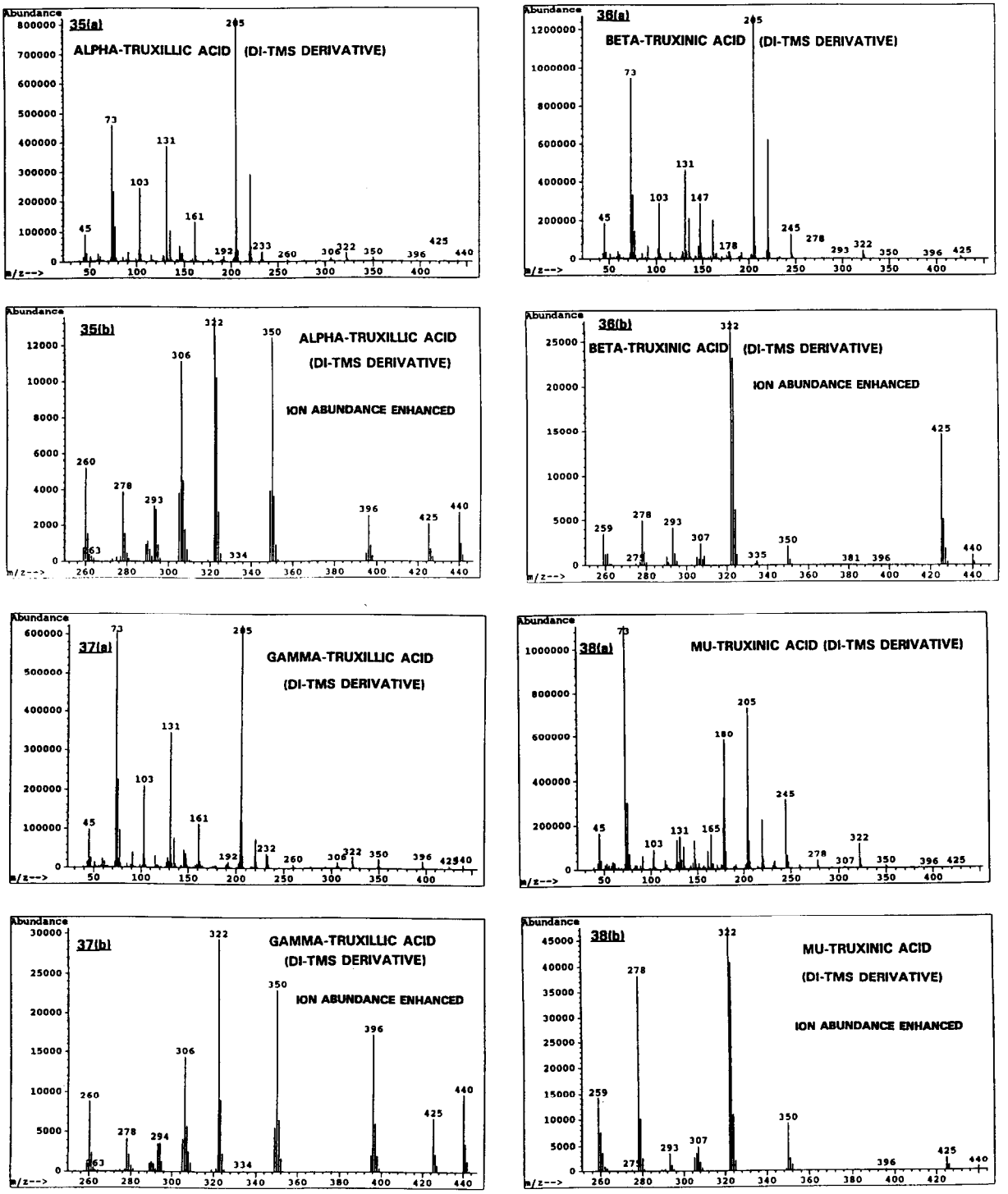


Fig. 16. *(Continued on p. 202)*

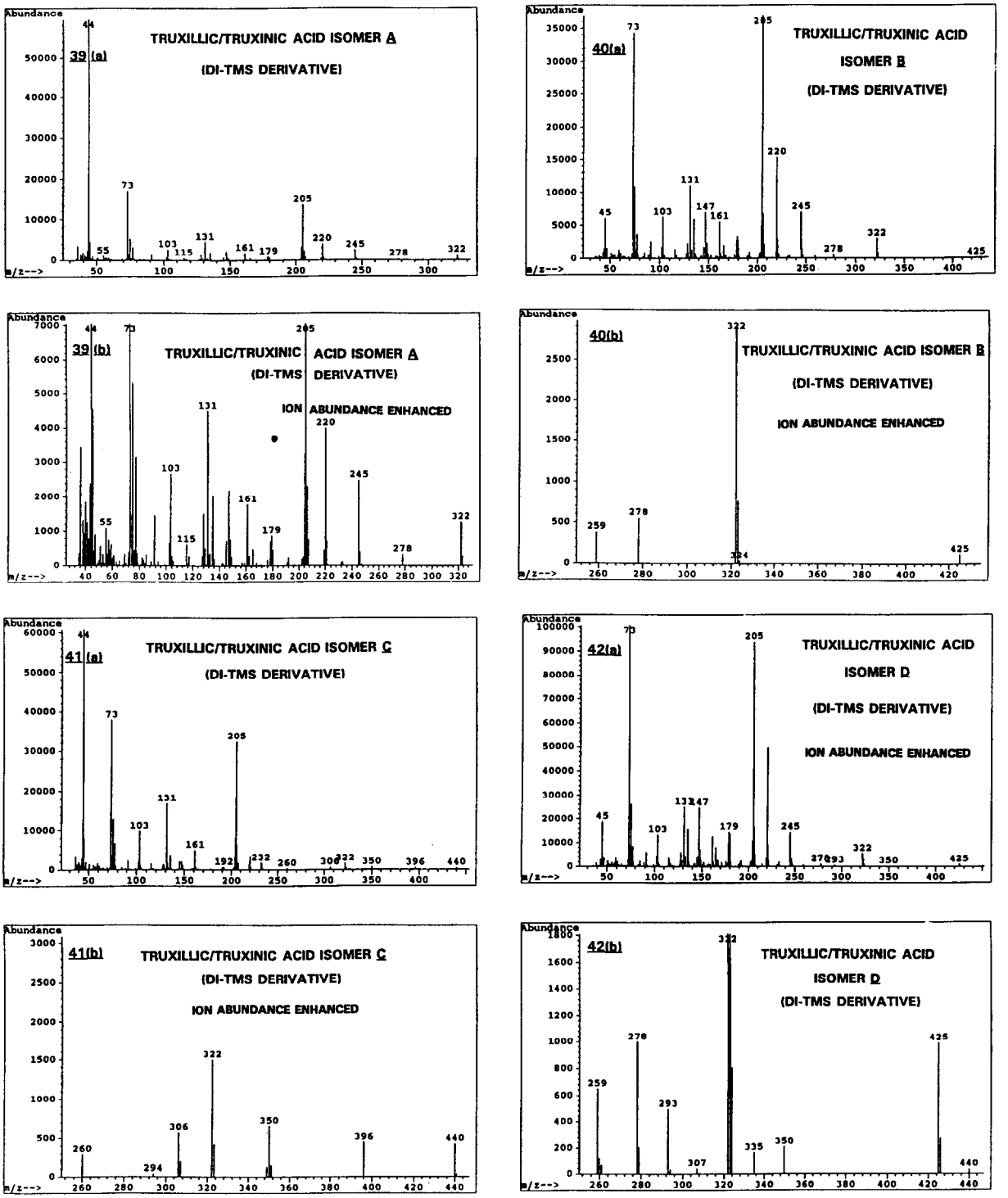

39 (a)
TRUXILLIC/TRUXINIC ACID ISOMER A
(DI-TMS DERIVATIVE)
40(a)
TRUXILLIC/TRUXINIC ACID ISOMER B
(DI-TMS DERIVATIVE)
39 (b)
TRUXILLIC/TRUXINIC ACID ISOMER A
(DI-TMS DERIVATIVE)
ION ABUNDANCE ENHANCED
40(b)
TRUXILLIC/TRUXINIC ACID ISOMER B
(DI-TMS DERIVATIVE)
ION ABUNDANCE ENHANCED
41 (a)
TRUXILLIC/TRUXINIC ACID ISOMER C
(DI-TMS DERIVATIVE)
42(a)
TRUXILLIC/TRUXINIC ACID ISOMER D
(DI-TMS DERIVATIVE)
ION ABUNDANCE ENHANCED
41(b)
TRUXILLIC/TRUXINIC ACID ISOMER C
(DI-TMS DERIVATIVE)
ION ABUNDANCE ENHANCED
42(b)
TRUXILLIC/TRUXINIC ACID ISOMER D
(DI-TMS DERIVATIVE)
Abundance
m/z-->

Fig. 16.

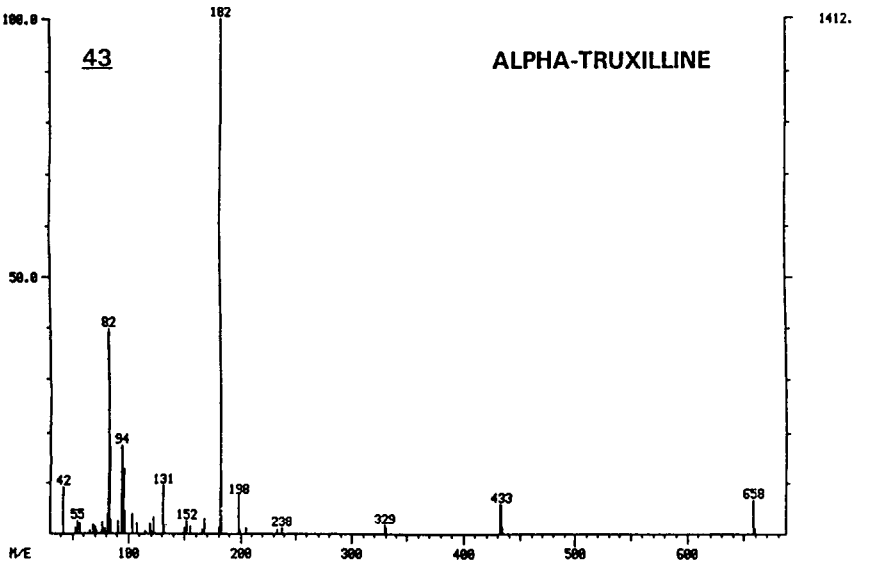


Fig. 16.

13. Acknowledgement

We are grateful to Donald A. Cooper for providing the electron ionization mass spectrum of α-truxilline.

14. Appendix: Electron ionization mass spectra of alkaloids and manufacturing by-products detected in the leaves of South American *E. coca* var. *coca* and/or illicit refined cocaine samples

All spectra (Fig. 16), excepting that for α-truxilline, were acquired on a Hewlett-Packard Model 5971 quadrupole mass-selective detector interfaced with a Hewlett-Packard 5890 Series II gas chromatograph. The mass-selective detector was operated in the electron ionization mode with an ionization potential of 70 eV, a secondary electron multiplier value of 1541 and 1.2 scans/s. The gas chromatograph was fitted with a 30 m $\times$ 0.25 mm I.D. fused-silica capillary column coated with DB-1 at a film thickness of 0.25 μm. The mass spectra of impurities/by-products with labile protons were also acquired as their trimethylsilyl derivatives. Derivatized sites include carboxyl, hydroxyl and secondary amine moieties. The spectra of four isomeric hydroxycocaines, as trimethylsilyl derivatives (mass spectra 31–34), were acquired from the cGC–MS of a concentrated coca leaf extract. Whereas the hydroxy groups of these isomers can be assigned to the tropane moiety (*e.g.*, see Fig. 8), their positional assignments are equivocal. The spectra of the isomeric truxillic/truxinic acids A–D, as their di-trimethylsilyl derivatives (seen in mass spectra 39–42), are represented by some of the structures illustrated in Fig. 4 (except the α, β, γ and μ isomers).

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