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Detection, Isolation, and Identification of Truxillines in Illicit Cocaine by Means of Thin-Layer Chromatography and Mass Spectrometry

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ABSTRACT: By means of thin-layer chromatography, an unidentified alkaloidal fraction was observed in illicit cocaine. Because of its persisting presence, efforts were undertaken to isolate and identify this fraction. Various analytical techniques showed complex results, finally pointing to the possibility of isomerization of constituents of the fraction. A direct-probe mass spectrum showed a fragmentation pattern which could only fit a truxilline. When using thin-layer chromatography, at least five isomers could be observed. This is the first time truxillines have been observed, isolated, and identified by thin-layer chromatography.

KEYWORDS: toxicology, cocaine, chromatographic analysis, thin-layer chromatography, truxillines

Thin-layer chromatography (TLC) is one of the most widely used methods for qualitative analysis of cocaine. It has retained favor as an analytical method primarily because of its simplicity, reliability, low cost, and selectivity of detection through the use of various location procedures [*1*]. The use of silica gel as the stationary phase is commonly preferred. Very limited applications have been described on aluminum oxide, cellulose, or kieselguhr.

Using TLC with silica gel as the stationary phase, Wartmann-Hafner [*2*] tested several solvent systems in order to separate cocaine, cinnamoylcocaine, tropacocaine, benzoylecgonine, and ecgonine. Ethyl acetate/ethanol/dimethylformamide/diethylamine (75:20:5:2) was used to separate cocaine from its accompanying substances, while the more polar substances were separated by methanol/diethylamine (95:5). Separation of cocaine and cinnamoylcocaine seemed rather difficult to achieve. Munier and Drapier [*3–5*] investigated a number of neutral solvent systems on silica gel plates, combining different chlorinated hydrocarbons with methanol. They observed that the selectivity of the system was determined by the chlorinated hydrocarbon, while increasing amounts of methanol produced increased mobility of all alkaloids. Samples of illicit cocaine are often adulterated with synthetic local anesthetics and their separation is not easy to achieve. Brown et al. [*6*] attempted to separate these substances with a mixture of ethyl acetate/*n*-pro-

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panol/28% ammonium hydroxide solution (40:30:3) on silica gel. A different approach was made by Tandon [7], who used five different solvent systems in order to identify cocaine, together with several other compounds that may be used as adulterants of illicit cocaine.

During TLC analysis of illicit cocaine samples, detection is usually performed by the quenching of ultraviolet (UV) light on the fluorescent plates at 254 nm, followed by spraying with Dragendorff reagent or iodoplatinate reagent. Although iodoplatinate seems to be more selective because of the differences in color obtained for various alkaloids, it is less sensitive than Dragendorff reagent. The sensitivity of the latter can be further enhanced by subsequent spraying with 20% sulfuric acid [9].

In our laboratories, two TLC systems are routinely used for the detection of cocaine, its congeners, and possible adulterants. This is a part of the forensic analysis on samples suspected of containing cocaine that are received from the police or customs authorities. By combining the information obtained from the neutral system chloroform/methanol (90:10 v/v) (System A) with the results of the basic system consisting of cyclohexane/toluene/diethylamine (75:15:10 v/v) (System B), cocaine could be identified in almost all cases, whereas adulteration (mostly with caffeine or lidocaine) was infrequently seen. Analysis of these "uncut" cocaine samples often indicated the presence of various by-alkaloids, and the identification of cinnamoylcocaine and tropacocaine could be confirmed using reference substances. Although the cocaine breakdown products benzoylecgonine and ecgonine are too polar to be chromatographed properly in either one of the TLC systems, a small flame-like spot just above the starting point may indicate the presence of benzoylecgonine when the plate is developed in System A. The TLC behavior of the above substances is summarized in Table 1.

During the past few years in Curaçao, Netherlands Antilles, thousands of illicit cocaine samples were preliminarily identified using the two mentioned TLC systems.

During the course of these investigations, we noticed that various samples contained an unknown spot, with an Rf value of about 0.30 in System B, whereas, in some cases, even two spots could be seen in this area. Although these spots were usually not detected during exposure of the plate to UV light of 254 nm, subsequent spraying with Dragendorff reagent visualized them as orange spots. However, the presence of these spots could not be confirmed in System A. Therefore, attempts were undertaken to isolate and identify these unknown alkaloids.

Materials and Methods

Solvents

All the solvents used were "Baker Analyzed" reagent grade (J. T. Baker Chemical Co., Phillipsburg, New Jersey), with the exception of diethylamine, which was reagent grade from Sigma (Sigma Chemical Co., St. Louis, Missouri).

Thin-Layer Chromatography

TLC was performed using 20 by 20-cm silica gel 60 F-254 precoated TLC plates, as well as 20 by 5-cm silica gel 60 F-254 precoated plates (Merck, Darmstadt, Germany). The plates were developed in saturated chambers. Saturation was achieved by using a double paper lining and a 1-h saturation time. The environmental temperature during the experiments was between 21 and 25°C and the relative humidity was 75 to 95%. For the spotting of the samples, 5 μL capillary columns were used (Drummond Scientific Co., Broomall, Pennsylvania), while the development distance was normally 15 cm. Two different types of development solutions were used: chloroform/methanol (90:10 v/v) (Solution A) and cyclohexane/toluene/diethylamine (75:15:10 v/v) (Solution B). Detec-

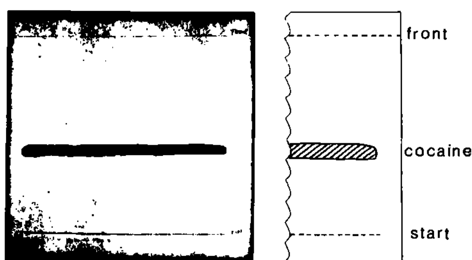

FIG. 1—*Thin-layer chromatogram of an illicit cocaine sample after elution with System B, photographed in UV light of 254 nm. The quenching zone of cocaine can be clearly observed. The drawing at the right side is meant as an illustration, in order to increase the clarity.*

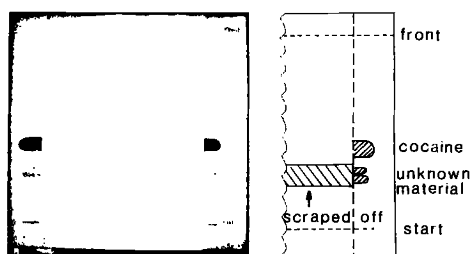

FIG. 2—*Thin-layer chromatogram of an illicit cocaine sample after elution with System B, photographed after spraying the uncovered ends with Dragendorff reagent. The drawing at the right side is meant as an illustration in order to increase the clarity.*

tion was done using UV light of 254 nm, followed by spraying with Dragendorff reagent. (Dragendorff reagent was prepared by mixing, immediately before use, equal volumes of Dragendorff Solutions A and B, which were purchased from Brinkmann Instruments Inc. (Westbury, New York). Dragendorff Solution A consisted of 0.94% bismuth subnitrate in 30.6% acetic acid, and Dragendorff Solution B was an 11.21% solution of potassium iodide in water.)

Gas Chromatography

GC analyses were performed using a Philips PU 4500 chromatograph (Philips/Pye Unicam, Cambridge, United Kingdom) fitted with dual flame ionization detectors. The results were processed with a Beckman 427 integrator (Beckman Instruments Inc., San Ramon, California). A 5-ft (1.5-m) glass column of 2.0-mm inside diameter was used, packed with 3% OV-1 on Chromsorb WHP (100 to 120 mesh) (Chrompack, Middelburg, The Netherlands). The nitrogen carrier gas flow rate was 30 mL/min. The flow rates of the detector gases were 30 mL/min for hydrogen and 300 mL/min for air. The injector and detector temperatures were maintained at 200 and 350°C, respectively. The oven temperature was held at 100°C for 2 min, then raised by 12°C/min to 320°C and held for 5 min.

Ultraviolet Spectrometry

UV spectra of solutions in 1-cm quartz cells were taken from 350 to 200 nm on a Philips PU 8800 UV/VIS spectrophotometer (Philips/Pye Unicam, Cambridge, United Kingdom).

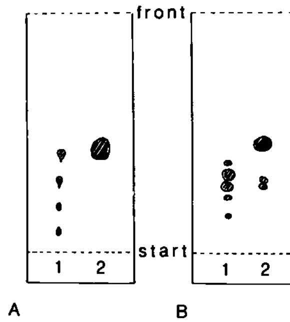

FIG. 3—*Thin layer chromatogram of the isolated zone (1) and the original cocaine sample (2), obtained after elution in Systems A and B, followed by visualization with Dragendorff reagent. See the text for details.*

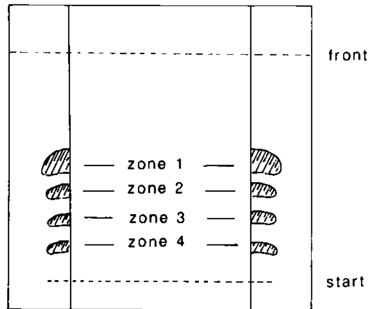

FIG. 4—*Schematic presentation of the unknown cocaine material after chromatography in System A. See the text for details.*

Gas Chromatography/Mass Spectrometry

GC/MS analyses were performed on a Finnigan MAT 212 mass spectrometer (Finnigan MAT, Bremen, Germany), connected to a Varian 3700 gas chromatograph (Varian, Palo Alto, California). Helium was used as the carrier gas, with a flow rate of 1.5 mL/min. A 25-m fused silica CP-Sil 5 CB capillary column 0.32 mm in inside diameter was employed. The injection temperature was set at 250°C. Following injection, the oven temperature was kept at 100°C for 2 min, then programed at a rate of 20°C/min to a final temperature of 280°C. The ion source and interface temperatures were 200°C. In the electron impact mode, the ionization energy was 70 eV. In the chemical ionization mode, ammonia was used as a reactant gas, and the ionization energy was 180 eV.

Mass Spectrometry

Direct-insertion-probe mass spectra [electron impact (EI)] at 70 eV were taken on a VG 70 SE double-focusing mass spectrometer in the high-resolution mode (R = 10 000) (VG Instruments, Wythenshawe, United Kingdom).

Reference Substances

Cocaine/hydrochloric acid (HCl) was obtained from Brocacef NV, Maarssen, The Netherlands; pseudococaine/HCl was obtained from Merck, Darmstadt, Germany). The

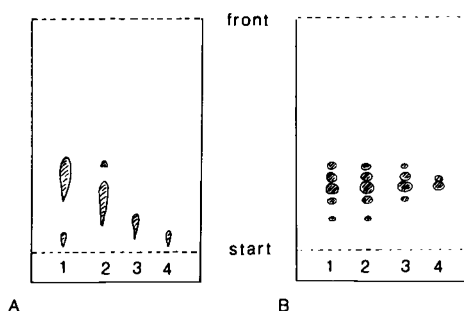

FIG. 5—*Chromatograms of the four isolated zones, chromatographed in Systems A and B, respectively. See the text for details.*

TABLE 1—*Thin-layer chromatographic behavior and visualization of cocaine, its by-alkaloids, and some adulterants.*

Substance	Rf Value in System A	Rf Value in System B	UV-254 nm Absorption	Dragendorff Reaction
Cocaine	0.47	0.47	positive	orange
Cinnamoylcocaine	0.47	0.42	positive	orange
Tropacocaine	0.22	0.34	positive	orange
Benzoylecgonine	0.01	0.00	positive	orange
Ecgonine	0.00	0.00	negative	orange/red
Caffeine	0.58	0.03	positive	no reaction
Lidocaine	0.71	0.35	positive	orange

allococaine and allopseudococaine were a gift from H. Huizer (Forensic Science Laboratory, Rijswijk, The Netherlands). Tropacocaine/HCl was obtained from Aldrich Chemie, Brussels, Belgium. *Trans*-cinnamoylcocaine, benzoylecgonine, and ecgonine were donated by the U.S. Drug Enforcement Administration (Special Testing and Research Laboratory, McLean, Virginia).

Results

In an effort to isolate the unknown material with an Rf value of $\approx$ 0.30 in TLC System B, 100 μL of a 5% chloroform solution of an illicit cocaine base sample was applied as a streak of 15 cm, followed by development in System B. The result is depicted in Fig. 1, which shows the quenching zone of cocaine, photographed in UV light of 254 nm, yet no quenching can be seen around Rf = 0.30. Next, the center of the plate was almost totally covered with filter paper, leaving small chromatographed zones to the left and right of the filter paper uncovered, and these free zones were sprayed with Dragendorff reagent. The filter paper was removed immediately thereafter and the unsprayed part of the silica gel, containing the unknown material—as indicated by the orange-colored spots at both ends—was scraped off (Fig. 2).

After grinding, the silica gel was transferred into a Pasteur pipette, closed at the narrow end with a cotton plug. Using 3 mL of a mixture of chloroform/methanol (50:50), the substance was eluted from the silica gel into a 5-mL sample vessel, and carefully evaporated to dryness under a stream of nitrogen. The residue was reconstituted in ~100 μL of chloroform, and 5-μL samples of this solution were spotted on two different TLC plates (5 by 20 cm each), together with a 5-μL sample of the original cocaine solution as a reference. One plate was chromatographed in System A and the other in System B.

After the plates had been developed, dried, viewed under UV light, and sprayed with Dragendorff reagent, a result was obtained, as depicted in Fig. 3. The isolated zone of Plate B now showed two closely eluting major spots at Rf values 0.27 and 0.32, respectively, accompanied by a series of minor spots. Plate A indicates for the same zone at least four different spots, which are almost unnoticeable under UV light of 254 nm but clearly visible after spraying.

It should be noted that in the drawings of the TLC plates, the spots do not reflect the intensities observed in actuality. In general, the spots in System A had a rather low intensity in comparison with those in System B.

In view of the separation properties of System A and System B, five isolations of the unknown zones were performed in System B, as described above. The combined eluates were evaporated to dryness and the residue was redissolved in 200 μL of chloroform. The latter was then applied as a streak on a new silica gel plate and developed in System A over 15 cm. Detection and subsequent isolation of the separated zones was done as described before for System B. The result in System A is depicted in Fig. 4. After visualization with Dragendorff reagent, four main zones could be distinguished, which were numbered 1 to 4.

After isolation of the four zones, each of them was dissolved in 50 μL of chloroform. Ten microlitres of each solution were spotted on two different TLC plates, which were developed in Systems A and B, respectively, in order to check the isolation performance as well as the chromatographic behavior of the different components. System A showed substances with different Rf values, though complete separation was not achieved and some spots showed considerable tailing. System B gave rise to an almost identical chromatographic behavior of all four zones: there were two closely eluting main spots at Rf values 0.27 and 0.32, respectively, accompanied by a series of minor spots. The results are depicted in Fig. 5.

Thus, after separating four zones in System A, rechromatography of these individual zones in System B appeared to bring back the original pattern of two main spots accompanied by a series of minor ones. This may reflect interconversion (that is, isomerization) of the substances separated in System A.

Identification

In an effort to identify the "cluster" of substances observed in System B, an amount of material, obtained after five isolations from System B was reconstituted in 5 mL of anhydrous ethanol, and a scanning UV spectrum was taken from 350 to 200 nm. This spectrum is depicted in Fig. 6, showing typical benzenoid absorptions at 253, 259, and 263 nm, respectively, which indicates the presence of benzenoid moiety in the molecule.

Subsequently taken UV spectra showed that the benzenoid absorption was present in ethanolic solutions of each of the isolated zones obtained after the separation in System A. The gas chromatogram of the isolated cluster from System B showed one broad peak, possibly due to more than one substance and eluting with an R_t of about 3 min. The same peak, according to retention time and peak shape was observed after injection of each of the four isolated zones from system A. Gas chromatography/mass spectrometry (GC/MS) analysis of the material isolated from System B in the chemical ionization (CI) mode indicated a quasi-molecular ion $(MH)^+$ at m/z 182, whereas the EI mode produced a fragmentation pattern that showed great similarity with earlier published mass spectra of anhydromethylecgonine [*9–11*]. The CI and EI mass spectra are given in Fig. 7; however, the latter component is devoid of a benzenoid moiety, whereas our unknown material shows the characteristic benzenoid UV absorption, as shown in Fig. 6.

Lukaszewski and Jeffery [*9*] identified anhydromethylecgonine as being a breakdown product of cocaine during GC analysis. Although this observation could not be confirmed

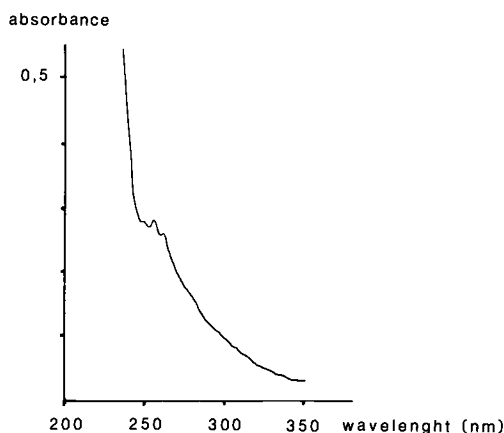

FIG. 6—*UV spectrum of the unknown material, isolated from System B.*

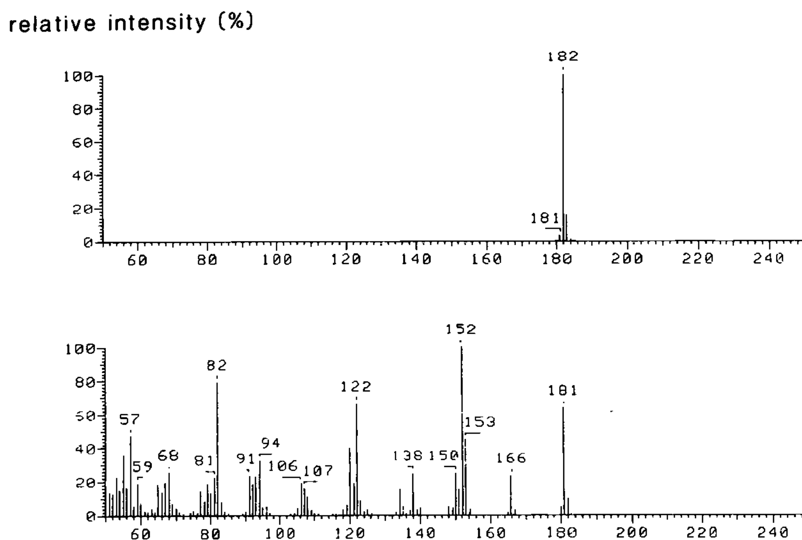

FIG. 7—*CI* (top) *and EI* (bottom) *mass spectra of the unknown material.*

in our laboratory, anhydromethylecgonine was prepared according to the method described by Lukaszewski, in order to compare it with the unknown substance, using different chromatographic techniques. Its structure was confirmed by means of GC/MS at the Forensic Science Laboratory, Rijswijk, The Netherlands. When comparing the chromatographic behavior of anhydromethylecgonine (AME) with our unknown substances using TLC (Systems A and B), no similarity was observed as can be seen in Fig. 8.

Lewin et al. [*12*] discussed the problems which may arise during gas chromatography (GC) analysis of the isomeric cocaines. Allococaine and allopseudococaine, in particular, eliminate benzoic acid very easily and the remaining methylecgonines are subsequently dehydrated to anhydromethylecgonine. Lewin et al. also mentioned the differences in retention times observed for anhydromethylecgonine arising from allococaine and allopseudococaine, respectively.

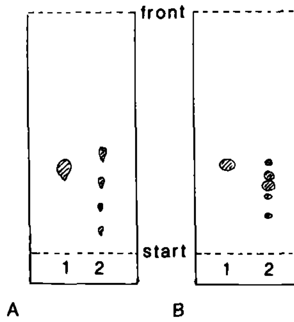

FIG. 8—*Thin-layer plates developed in Systems A and B, respectively: (1) AME, (2) unknown substance.*

Indeed, the gas chromatograms of all the isomeric cocaines showed retention time variations, not only between the cocaines, but also between their respective breakdown products (AME). When mixtures of the isomers were injected, separation of the cocaines was not established, and also their breakdown products coeluted, giving rise to just one peak. However, thin-layer chromatography, using both System A and System B, provided better separation of the isomeric cocaines, as can be seen in Fig. 9. Yet, the unknown material did not show similarity with AME, neither in terms of Rf value nor in UV absorption, nor in the visualization with Dragendorff reagent. In the latter, AME gave an orange color that quickly faded away, whereas the orange color given by the unknown material was quite stable.

Thus, the above experiments indicate that the unknown material in illicit cocaine differs from the known cocaine isomers, analogues, and metabolites. When exposed to GC, it yields AME due to thermal degradation. Therefore, a direct-probe EI mass spectrum of the unknown material was taken and the result is depicted in Fig. 10.

With a molecular ion at *m/z* 658 and characteristic fragments at *m/z* 477, 433, 329, 182, and 82, the unknown material could be tentatively identified as one—or more—truxilline isomers, the general structures of which are given in Fig. 11.

The mass spectrum interpretation of the unknown material as being truxilline is supported by the earlier mentioned benzenoid UV absorption and the described GC behavior, the latter resulting in the breakdown product AME. The TLC observations, indicating the possible existence of at least 5 isomeric compounds, can now be understood much better: based on the isomeric properties of truxillic acid and truxinic acid, 15 isomers may exist, and it is conceivable that isomerization of isolated individual truxillines may occur. Recently, Moore et al. [*13*] were able to indirectly identify 5 truxilline isomers in

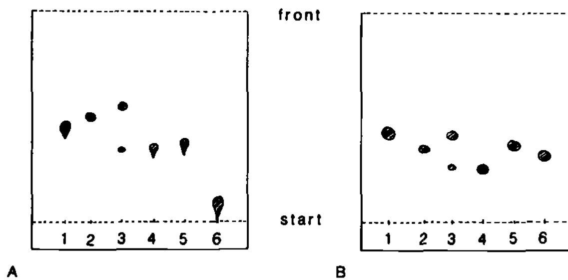

FIG. 9—*Thin-layer chromatograms of the isomeric cocaines, AME and methylecgonine, developed in Systems A and B, respectively: (1) cocaine, (2) pseudococaine, (3) allococaine, (4) allopseudococaine, (5) anhydromethylecgonine, (6) methylecgonine.*

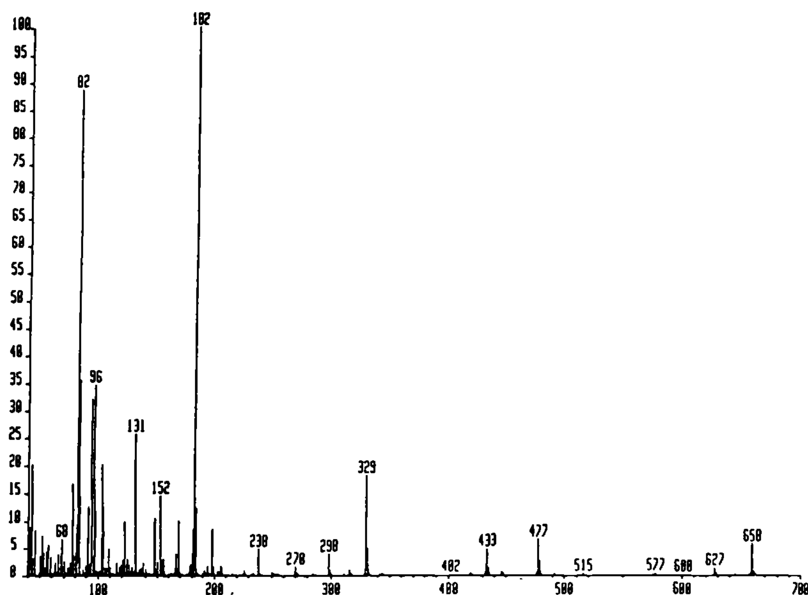

FIG. 10—*Direct probe EI mass spectrum of the unknown material.*

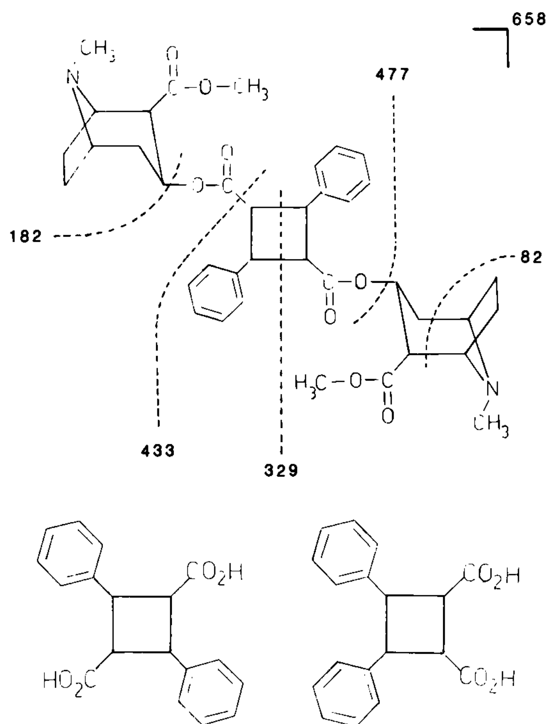

FIG. 11—*Generalized structure of a truxilline, based on truxillic acid. The fragments observed in mass spectrometry are indicated by dotted lines. Truxillines can be based on truxillic acid* (bottom left) *or on truxinic acid* (bottom right).

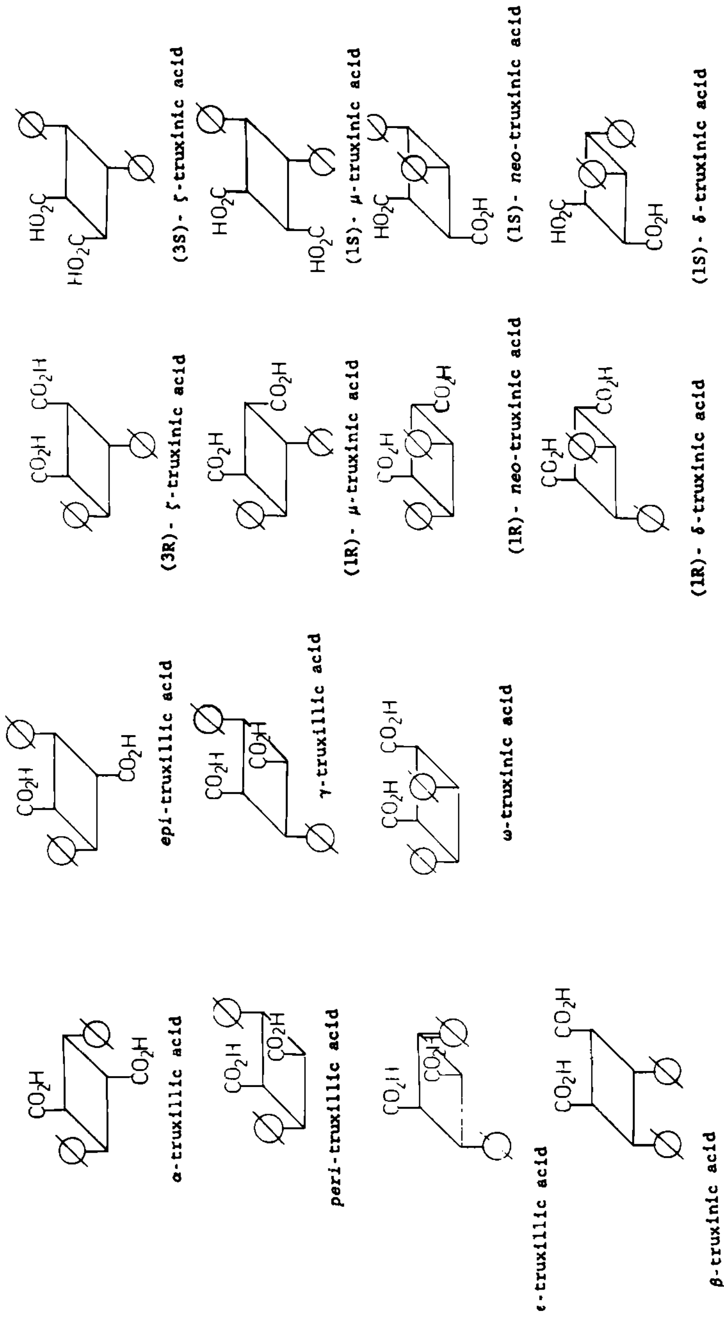

FIG. 12—*Possible structures of truxillic and truxinic acids.*

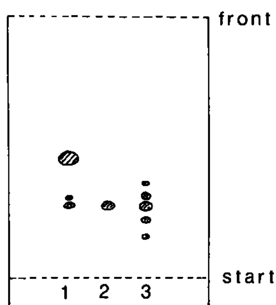

FIG. 13—*TLC chromatogram of an illicit cocaine sample (1), α-truxilline (2) and the "unknown" material (3), after development in System B, and visualization with Dragendorff reagent.*

extracts of coca leaves and illicit cocaine samples using GC, namely, the α, β, γ, δ, and ε isomers. They mentioned that 6 other isomers could exist—namely, *epi-*, *peri-*, *neo-*, μ-, ω-, and ζ- truxilline—for which they had reasonable evidence. They also found that the truxillines can easily decompose in GC to yield AME.

In our opinion, however, there are 15 possible isomers, because the *neo-*, ζ, μ, and δ truxinic acids can occur in enantiomeric forms. These 15 configurations of the acids are schematically given in Fig. 12. The nomenclature is according to Uff [*14*].

When preparing this paper, a reference sample of α-truxilline was kindly made available to us by the U.S. Drug Enforcement Administration, Special Testing and Research Laboratory, McLean, Virginia, through the courtesy of S. Sobol.

Further experiments revealed that α-truxilline had the same Rf value as the major truxilline spot in our TLC System B (see Fig. 13) and the upper zone in TLC System A, used for the isolation (see Fig. 4).

Discussion

At the end of the past century the possibility that truxillines could exist in extracts of coca leaves was recognized by Hesse [*15*] and Liebermann [*16*]. Both scientists reported the presence of α-truxillic and β-truxinic acids in hydrolysates of coca leaves. The corresponding alkaloids were named α- and β-truxilline. Their research was later corroborated by Hardy [*17*], who used a modified Vitali reaction in order to demonstrate the presence of the intact truxillines in cocaine samples. Fikenscher [*18*] gave additional indirect evidence for the possible existence of the truxillines in coca leaves by paper chromatography identification of α-truxillic and β-truxinic acid after hydrolysis.

Although thin-layer chromatography has been—and still is—widely used in the analysis of coca leaf preparations and illicit cocaine samples, observations of the possible presence of truxillines have not been mentioned in the literature before. This may partially be due to the low amounts of truxillines in the illicit samples. On the other hand, numerous TLC systems do not provide adequate separation between cocaine and the truxilline "cluster" or between the isomeric truxillines themselves. Using System B as the eluent, separation and detection of at least a few of the isomers was achieved. To the best of our knowledge, this is the first time the truxillines have been detected and identified with TLC. When this research was in progress, Moore et al. [*13*] were the first to provide definitive proof that truxillines occur as such in coca leaves. They separated them by means of capillary gas chromatography and electron capture detection after reduction and derivatization of the samples. In their work, the presence of at least five isomeric truxillines in coca leaf and illicit cocaine samples was established. Using TLC, we were able to isolate and finally identify the variable, yet persisting, presence of some of the

isomeric truxillines in illicit cocaine. These compounds are apparently formed in the leaves of the coca bush, together with small amounts of tropacocaine and *cis*- and *trans*-cinnamoylcocaine as by-alkaloids. It is not likely that they are formed during the extraction process, or that they will be affected by a possible oxidation with potassium permanganate, which is often performed by the illegal cocaine manufacturers in order to "bleach" the cocaine (cinnamoylcocaine and other unsaturated substances may be oxidized), so their presence may indicate the natural origin of the cocaine. After analyzing more than 5000 cocaine samples in recent years, we found that the presence of low, but variable, amounts of truxillines was confirmed in almost all cases when using TLC with System B, followed by spraying with Dragendorff reagent.

It will be interesting to investigate to what extent the presence of the truxillines can be utilized to establish the geographical origin of illicit cocaine samples and the type of processing that they have undergone. This may be an important and fruitful area for further research.

Acknowledgments

This work was performed at the Government Laboratory for Public Health, Willemstad, Curaçao, Netherlands Antilles, during 1982 through 1989. We are grateful to Dr. J. M. Eustatia, laboratory director, for providing the facilities and for his support of the project. We would like to thank Dr. L. Maat (Department of Organic Chemistry, Technical University, Delft, The Netherlands) for his support with the direct probe mass spectrum; G. T. Nagel (Central Clinical Chemical Laboratory, University Hospital, Groningen, The Netherlands) for performing the GC/MS analysis; and S. Sobol (Special Testing and Research Laboratory, McLean, Virginia) for the gift of a sample of α-truxilline.

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