Identification of *cis*- and *trans*-Cinnamoylcocaine in Illicit Cocaine Seizures

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During the in-depth analysis of illicit cocaine samples small amounts of other coca alkaloids and cocaine degradation products have been detected. One of these alkaloids, cinnamoylcocaine, has been found in more than half of the samples examined, usually in concentrations of 1% or less of the amount of cocaine present. The presence of cinnamoylcocaine, as its *cis* and *trans* isomers, was established by column partition chromatographic isolation of the isomers, followed by ultraviolet, infrared, nuclear magnetic resonance, and mass spectrometric identification.

Although cocaine hydrochloride is used in legitimate medicine as a topical anesthetic it also has widespread use as a stimulant in the illicit drug market. Most of the illicit cocaine used in the United States is believed to originate from the South American coca plant. Cocaine is extracted from the coca leaf and subsequently purified in clandestine laboratories located in the coca-producing areas.

In addition to cocaine, the presence of other alkaloids in the coca leaf has been established (1–4). Some of these include methylecgonine, tropacocaine, truxilline, cinnamoylcocaine, and cuscohygrine. However, cocaine is the substance of most significance and usually constitutes the major portion of the ecgonine-based alkaloids of the leaf. Substances such as ecgonine, benzoylecgonine, pseudococaine, and cocaethyline have been detected in cocaine and are believed to be produced primarily during the manufacturing process (1, 4–7). It is interesting to note that, in most studies on the coca alkaloids, the isomeric purity of cinnamoylcocaine is not discussed. In addition, most of these studies have dealt with the coca leaf, biological specimens, and cocaine pharmacopoeial standards. Very little work has been published on the detection of these substances in illicit cocaine seizures.

The presence of cinnamoylcocaine and other alkaloids in cocaine is a reflection on the quality of the illicit manufacturing procedures used. For reasons which will be discussed later, methodology utilizing silyl derivatization and gas-liquid chromatography (GLC) has been developed to detect and, in some cases, quantitate ecgonine, benzoylecgonine, methylecgonine, cocaethyline, tropacocaine, ethylecgonine, pseudotropine, and cinnamoylcocaine in seizures of illicit cocaine. While some of these substances have been detected and quantitated in a number of samples, cinnamoylcocaine appeared to be one of the most frequent contaminants detected.

The ability to detect and quantitate small amounts of alkaloids and degradation products in large quantities of clandestinely produced narcotics, such as cocaine and heroin, can be of value for several reasons. First, when developing analytical methodology to quantitate illicit narcotic substances such as cocaine, the forensic chemist should be aware of related substances that could affect the accuracy of the analysis. It has been shown that, in addition to cinnamoylcocaine, other cocaine-related substances are sometimes present which can affect quantitative results (J. M. Moore, 1972, unpublished data).

Second, the detection of substances such as cinnamoylcocaine provides information relative to the type of clandestine procedure used to manufacture cocaine. One method for cocaine production involves extraction of the coca leaf followed by successive recrystallizations. In the other procedure all of the ecgonine-based alkaloids of the leaf are hydrolyzed to ecgonine, which in turn is the starting material for the chemical synthesis of cocaine. The presence of cinnamoylcocaine in a given sample is a strong indication that the first procedure was used, since the hydrolysis step in the synthetic method would yield cinnamic acid instead of cinnamoylcocaine. Since the chemical synthesis method would require somewhat more "sophisticated" equipment and reagents, this information would be useful to enforcement officials in their efforts to eliminate clandestine cocaine laboratories.

Last and perhaps most important, the ability

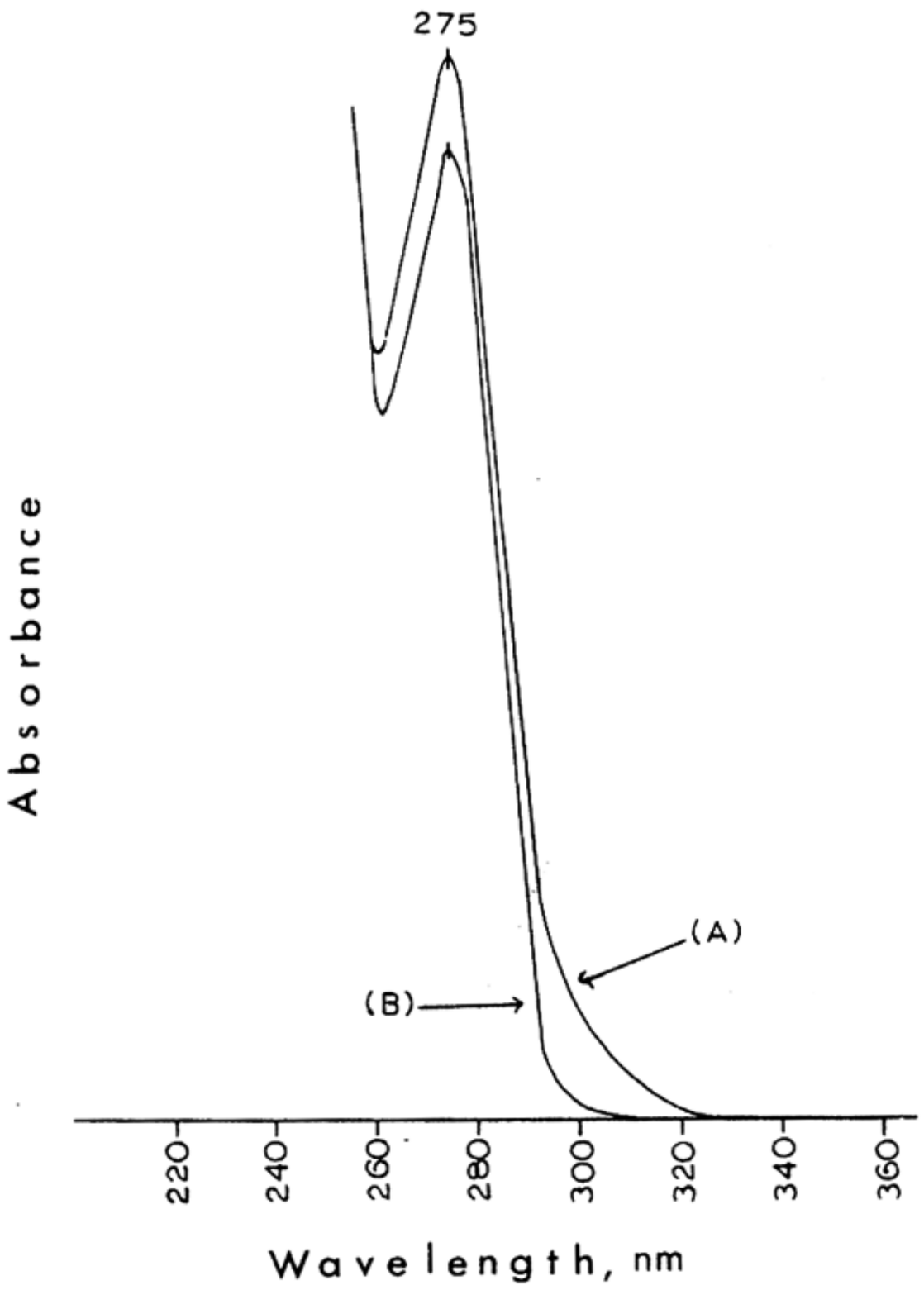

FIG. 1—Ultraviolet spectra of (A) illicit cocaine seized in Sicily and containing small amounts of suspected cinnamoylcocaine; (B) about 0.25 mg USP Cocaine HCl/ml dilute sulfuric acid, with absorbance of 0.695.

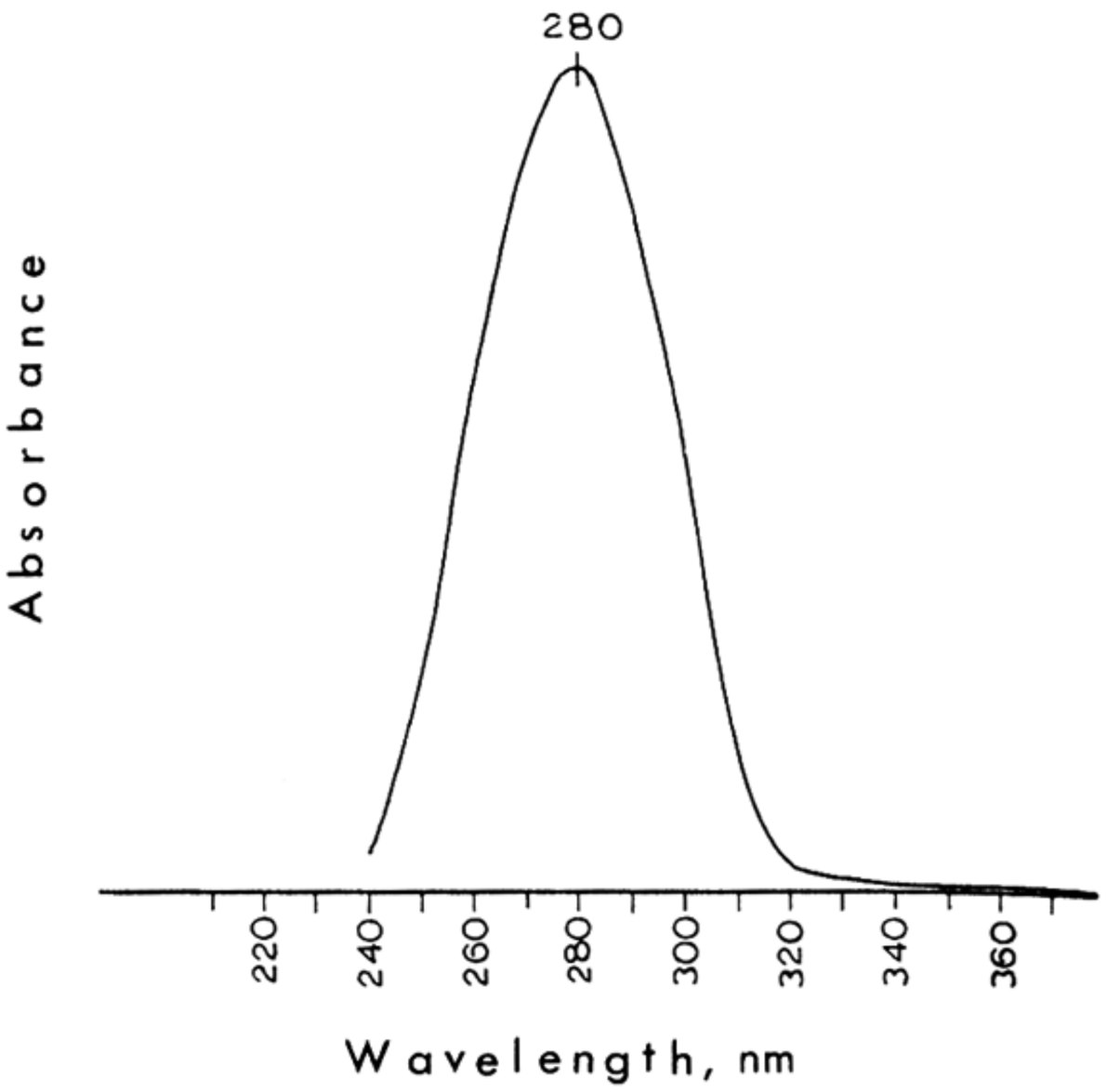

FIG. 2—Ultraviolet spectrum of 0.010 mg standard *trans*-cinnamoylcocaine/ml dilute sulfuric acid.

to detect and quantitate minor alkaloids in cocaine seizures may allow comparison of any number of cocaine seizures to determine if they are from a common origin. Developing this capability could aid enforcement officials in tracing international as well as domestic cocaine distribution routes and perhaps assist in narcotic conspiracy cases.

The presence of cinnamoylcocaine in illicit cocaine seizures had been suspected because of the following observations:

(*1*) The ultraviolet (UV) scan of illicit cocaine hydrochloride aqueous solutions between 350 and 220 nm revealed an inflection at about 300 nm which was not present in the spectrum of the USP cocaine hydrochloride standard solution (Fig. 1). This inflection interfered with cocaine quantitation at the 275 nm maximum but did not interfere with the 232 nm band. Examination of the UV spectrum of cinnamoylcocaine in dilute acid revealed a maximum absorbance at 280 nm while it absorbed minimally near the second cocaine maximum at 232 nm (Fig. 2).

(*2*) In the samples where the UV spectra indicated the presence of cinnamoylcocaine, GLC analysis (J. M. Moore, 1972, unpublished information) always revealed 2 peaks eluting after cocaine (Fig. 3). The retention time of either peak, relative to cocaine, was consistent with the melting point and other physical properties of cinnamoylcocaine. The presence of 2 peaks indicated the possible decomposition of cinnamoylcocaine in the gas chromatograph, the presence of geometrical isomers, or the presence of a cinnamoylcocaine oxidation product (clandestine manufacturers use a permanganate compound to oxidize certain coca leaf constituents).

(*3*) The samples that contained suspected cinnamoylcocaine decolorized dilute solutions of potassium permanganate, indicating the possible presence of an olefinic bond. This permanganate treatment also caused the inflection on the 275 nm cocaine maximum to disappear. This was strong evidence that the olefinic bond was involved in a conjugated system, such as in cinnamolycocaine.

(*4*) Finally, mass spectrometric analysis of the contaminated cocaine samples produced what was believed to be a molecular ion at 329.2 mass units; interpretation of the entire mass spectrum was not possible, however, due to the overwhelming presence of cocaine hydrochloride.

To confirm absolutely the presence of cinnamoylcocaine and to establish its isomeric purity, the following steps were taken: (*1*) *trans*-Cinnam-

oylcocaine was synthesized from ecgonine, boron trichloride-methanol, and cinnamoyl chloride. (*2*) Suspected *cis*- and *trans*-cinnamoylcocaine was isolated from a crude bi-alkaloidal extract of the coca leaf by column partition chromatography. (*3*) The suspected *trans*-cinnamoylcocaine partition chromatographic fraction was identified by comparing its UV, infrared (IR), nuclear magnetic resonance (NMR), and mass spectral data with the synthesized *trans*-cinnamoylcocaine. (*4*) Identification of the suspected *cis*-cinnamoylcocaine partition chromatographic fraction was completed by the techniques mentioned for *trans*-cinnamoylcocaine.

Following the identification of the suspected cinnamoylcocaines, these steps were taken: (*1*) A comparison was made of the gas chromatographic retention times of the identified cinnamoylcocaines with the peaks found in illicit cocaine samples (Fig. 3); (*2*) authentic coca leaves were extracted in an attempt to isolate any cinnamoylcocaines present.

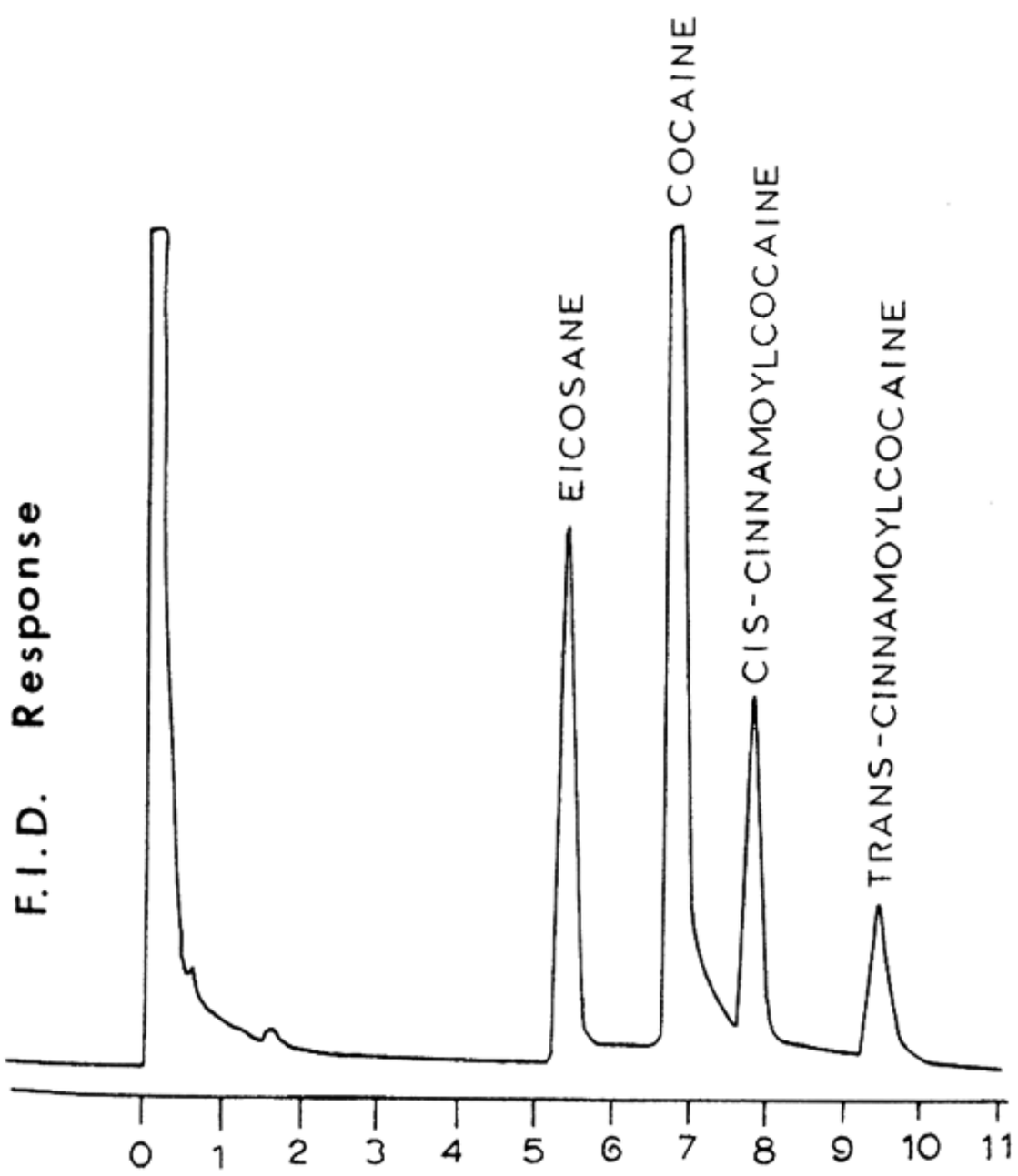

FIG. 3—Gas chromatogram of illicit Peruvian coca paste after silyl treatment using eicosane as an internal standard.

Gas chromatographic conditions: 4′ × ¼″ id coiled glass column packed with 3% OV-1 on 100–120 mesh Chromosorb W HP; column temperature was programmed with initial column temperature of about 190°C and an initial hold of 5 min with program rate of 20°/min; final temperature was 280°C with final hold of 10 min; injection and detector temperatures were 270 and 240°C, respectively; flame ionization detector was used with nitrogen carrier, hydrogen, and air flow rates of about 60, 50, and 500 ml/min, respectively.

Experimental

Standards

(a) *Cocaine hydrochloride.*—The standard used in this study was of USP quality (obtained from Merck and Co., Inc., Rahway, N.J. 07065).

(b) *trans-Cinnamoylcocaine.*—Synthesized by author as described below.

(c) *Eicosane internal standard.*—Applied Science Laboratories, Inc., P.O. Box 440, State College, Pa. 16801.

Reagents

(a) *Chloroform and N,N-dimethylformamide.*—Reagent grade (Matheson, Coleman & Bell, Raleigh, N.C. 27202).

(b) *Ethyl ether.*—Distilled-in-glass (Burdick and Jackson Laboratories, Inc., Muskegon, Mich. 49442).

(c) *Benzene.*—J. T. Baker Chemical Co., Phillipsburgh, N.J. 08865.

(d) *Sodium hydroxide.*—10% aqueous.

(e) *Sulfuric acid.*—1*N* aqueous.

(f) *Sodium carbonate and ammonium hydroxide.*—Reagent grade.

(g) *Hydrochloric acid.*—0.1*N* and 2*N* aqueous.

(h) *Boron trichloride-methanol.*—10% (w/v), Applied Science Laboratories, Inc.

(i) *Cinnamoyl chloride.*—Eastman Kodak Co., Rochester, N.Y. 14650.

Chromatographic Materials

(a) *Chromatographic columns.*—Glass, 22 × 300 mm, with 45 mm delivery stem (Kontes Glass Co., Vineland, N.J. 07360).

(b) *Column partition chromatographic solid support.*—Celite 545, acid-washed (Johns-Manville, Manville, N.J. 08835).

(c) *Gas chromatographic stationary phase.*—3% OV-1 on 100–120 mesh Chromosorb W HP (Applied Science Laboratories, Inc.).

Botanical Materials

(a) *Crude cocaine bi-alkaloid extract.*—Stepan Chemical Co., Maywood Division, Maywood, N.J. 07607.

(b) *Coca leaves.*—Obtained by agents of the Bureau of Narcotics and Dangerous Drugs from Bolivia.

Instrumentation

(a) *Gas chromatograph.*—Packard Model 7400 (for operating parameters, *see* Fig. 3).

(b) *Ultraviolet spectrophotometer.*—Cary Model 14.

(c) *Infrared spectrophotometer.*—Perkin-Elmer Model 457.

(d) *Nuclear magnetic resonance spectrometer.*—Joel Model C-60HL.

(e) *Mass spectrometer.*—Hitachi Model RMU-6L, single focusing.

Synthesis of trans-*Cinnamoylcocaine*

Dissolve 1–2 g standard cocaine HCl in ca 150 ml 1*N* HCl and boil this solution to volume of <20 ml. Cool solution and add acetone to crystallize ecgonine HCl. Filter or centrifuge solution and wash residue with several volumes of acetone and then $CHCl_3$. Dry residue (ecgonine HCl) under vacuum.

Dissolve ca 0.5 g ecgonine HCl in ca 25 ml boron trichloride-methanol. Heat solution ca 1 hr in loosely glass-stoppered test tube in 75°C water bath. Cool reaction solution, dilute to ca 50 ml with distilled water, and make basic with ammonia. Extract methylecgonine with several portions of $CHCl_3$, filtering each extract through cotton. Gently evaporate combined $CHCl_3$ extracts to ca 5 ml. Transfer $CHCl_3$ solution containing methylecgonine to 50 ml round-bottom reflux flask and add benzene to make total volume of ca 30 ml. Add 1 g cinnamoyl chloride[1] and 0.5 g anhydrous sodium carbonate to this solution and reflux overnight. After refluxing is complete, cool solution and transfer contents to separatory funnel. Add 25 ml ethyl ether and equal volume of 1*N* H_2SO_4 to separatory funnel, mix cautiously to neutralize sodium carbonate, and then mix vigorously to extract *trans*-cinnamoylcocaine and unreacted methylecgonine into acid layer. Extract acid layer with four 25 ml portions of $CHCl_3$, discarding these extracts. Make acid layer basic by slow addition of 10% NaOH and then extract with four 25 ml portions of $CHCl_3$. Extract combined $CHCl_3$ extracts with two 10 ml portions of 2*N* HCl. Extract $CHCl_3$ layer, now containing hydrochloride salt of *trans*-cinnamoylcocaine, once with equal volume of dilute ammonia solution. Filter $CHCl_3$ layer through cotton and evaporate to 2–3 ml. Add petroleum ether and cool solution to crystallize free base of *trans*-cinnamoylcocaine.

Isolation of cis- *and* trans-*Cinnamoylcocaine from Crude Coca Bi-Alkaloidal Extract by Column Partition Chromatography*

(Since cinnamoylcocaine in refined illicit cocaine samples is usually present in only 1% or less it was decided to work with a source richer in this alkaloid, namely, a crude coca bi-alkaloidal extract. It was noted that the 2 suspected cinnamoylcocaine GLC peaks of the illicit cocaine samples and 2 peaks in the crude bi-alkaloid extract had identical retention times.)

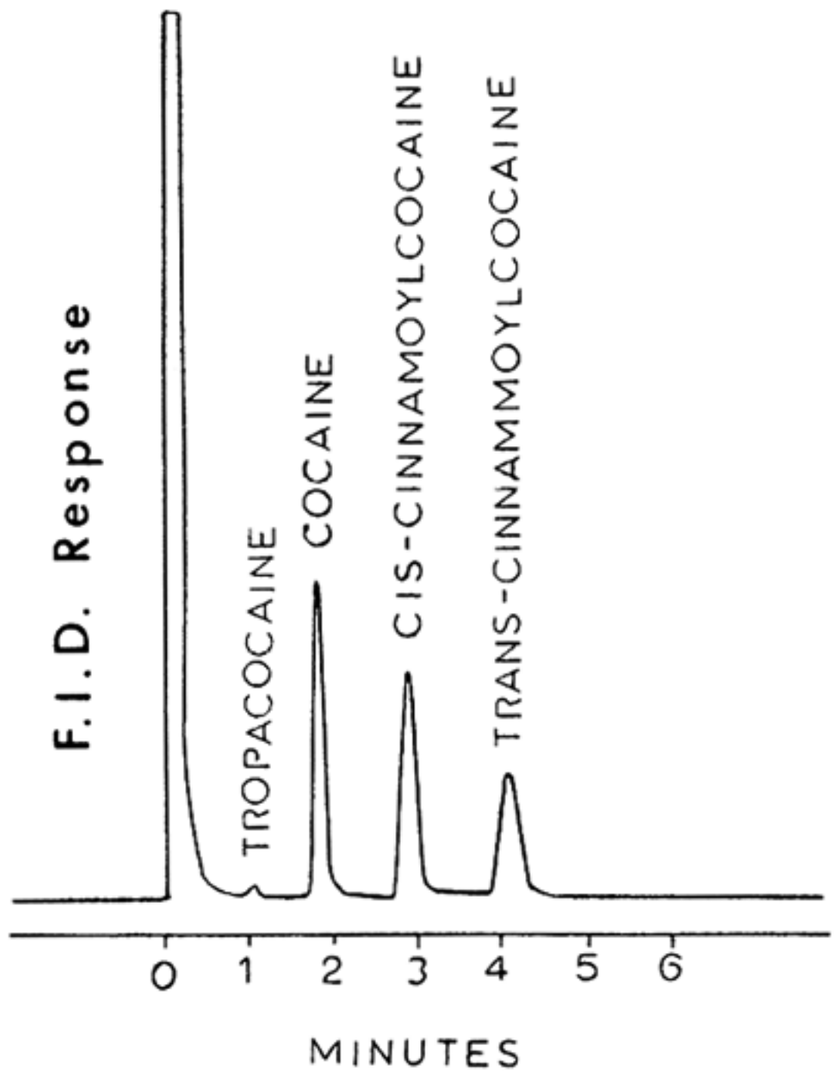

FIG. 4—Gas chromatogram of commercial crude bi-alkaloid after extraction and elution through a 2*N* HCl-Celite 545 chromatographic column with water-washed chloroform.

Gas chromatographic conditions: Parameters are the same as in Fig. 3 except the column temperature is operated isothermally at about 225°C.

Dissolve crude extract in $CHCl_3$ by warming gently and then extract with 0.5*N* H_2SO_4, discarding $CHCl_3$ layer. Make acid layer basic with ammonia and extract alkaloidal bases with $CHCl_3$. Evaporate combined $CHCl_3$ extracts to <10 ml and then decant onto chromatographic column packed with 5 ml 2*N* HCl and 8 g Celite 545. Elute column with water-washed $CHCl_3$ until ca 200 ml eluate is collected. (GLC analysis of this elution is illustrated in Fig. 4.) Gently evaporate $CHCl_3$ eluate to dryness on steam bath under air. Add 5 ml 0.1*N* HCl to residue and swirl to dissolve residue. Then add 7 g Celite and mix until fluffy. Pack on column containing mixture of 5 ml 0.1*N* HCl and 7 g Celite 545. Successively elute column with following water-washed solvents: 50 ml $CHCl_3$-ether (1+1), 50 ml $CHCl_3$-ether (3+1), 50 ml $CHCl_3$-ether (9+1), and finally ca 1 L $CHCl_3$. The eluate was collected in 25 ml fractions for monitoring by GLC under conditions given in Fig. 4. Column partition chromatographic elution pattern of tropacocaine, suspected *trans*- and *cis*-cinnamoylcocaine (compounds (B) and (C)), and cocaine is illustrated in Fig. 5. Nearly pure *trans*-cinnamoylcocaine and *cis*-cinnamoylcocaine were isolated by utilizing 225–275 ml and 575–625 ml fractions, respectively. Suspected cinnamoylcocaines in both fractions were converted to free bases prior to spectrometric identification.

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[1] This compound was determined to be primarily the *trans* isomer by aqueous hydrolysis of the acid chloride, followed by IR comparison with commercial *trans*-cinnamic acid. In addition, GLC analysis of the final product did not reveal any *cis*-cinnamoylcocaine.

This paper was presented at the Forensic Sciences Symposium, 86th Annual Meeting of the AOAC, Oct. 9–12, 1972, at Washington, D.C.

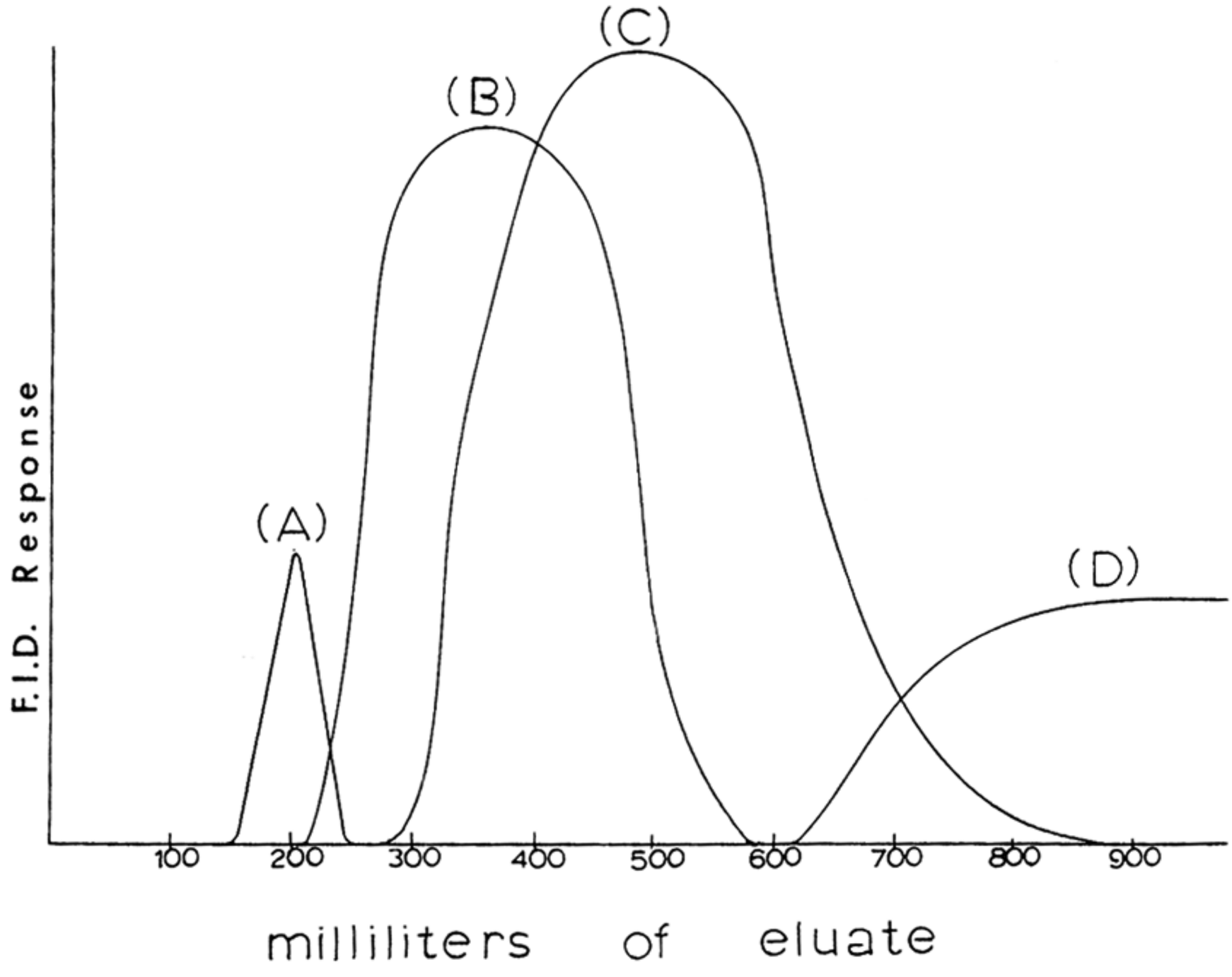

FIG. 5—Column partition chromatographic elution pattern of (A) tropacocaine, (B) *trans*-cinnamoylcocaine, (C) *cis*-cinnamoylcocaine, and (D) cocaine. The column eluate was monitored in 25 ml fractions by GLC under the parameters given in Fig. 4.

Discussion

Ultraviolet Analysis

The UV spectra of compounds (B) and (C) were the same as that for standard *trans*-cinnamoylcocaine between 350 and 220 nm. This stongly suggested the presence of a cinnamic acid chromophore in compounds (B) and (C). When the isolation procedure is considered, it is probable that 2 closely related cinnamoylcocaines were present. The possibility of either compound (B) or (C) being an oxidation product of cinnamoylcocaine was refuted by the UV findings, because oxidation, using a permanganate salt, would destroy the olefinic bond in cinnamoylcocaine, altering the UV spectrum markedly.

Mass Spectral Analysis

Compounds (B) and (C) and standard *trans*-innamoylcocaine yielded nearly identical mass pectra. These data supported the UV findings, amely, that both compounds were closely re-ated cinnamoylcocaines. Furthermore, both pecies yielded the same parent peak, which sug-ested that the cinnamoylcocaines were related in n isomeric manner. Since cinnamoylcocaine con-ains an olefinic bond, the relationship probably volved *cis* and *trans* isomerism around the ouble bond.

Infrared Analysis

Spectrometric evidence thus far suggested that compounds (B) and (C) were probably *trans*- and *cis*-cinnamoylcocaine, respectively. The IR spectra of (B) and (C) and standard *trans*-cinnamoylcocaine, all as free bases, were recorded between 4000 and 250 cm^{-1} (Fig. 6). The spectra of compound (B) and standard *trans*-cinnamoylcocaine were the same, thus confirming (B) as the *trans* isomer. The IR spectra of compounds (B) and (C) were then compared in an effort to determine if an isomeric relationship existed around the olefinic bond (8–11).

There was a marked difference between the 2 compounds in the absorption intensity of the C═C stretching frequency at about 1625 cm^{-1}. This suggested that compound (C) differed from (B) around the olefinic bond. However, the more intense 1625 cm^{-1} band of *trans*-cinnamoylcocaine was an apparent exception to the general observation that the C═C stretching intensities of transoid compounds are weaker than their cisoid counterparts.

Both compounds exhibited 2 carbonyl stretching bands due to substituents at C2 and C3 on the tropane molecule. Both compounds had carbonyl absorption at about 1745 cm^{-1} due to the C2 substituent. However, the carbonyl absorption

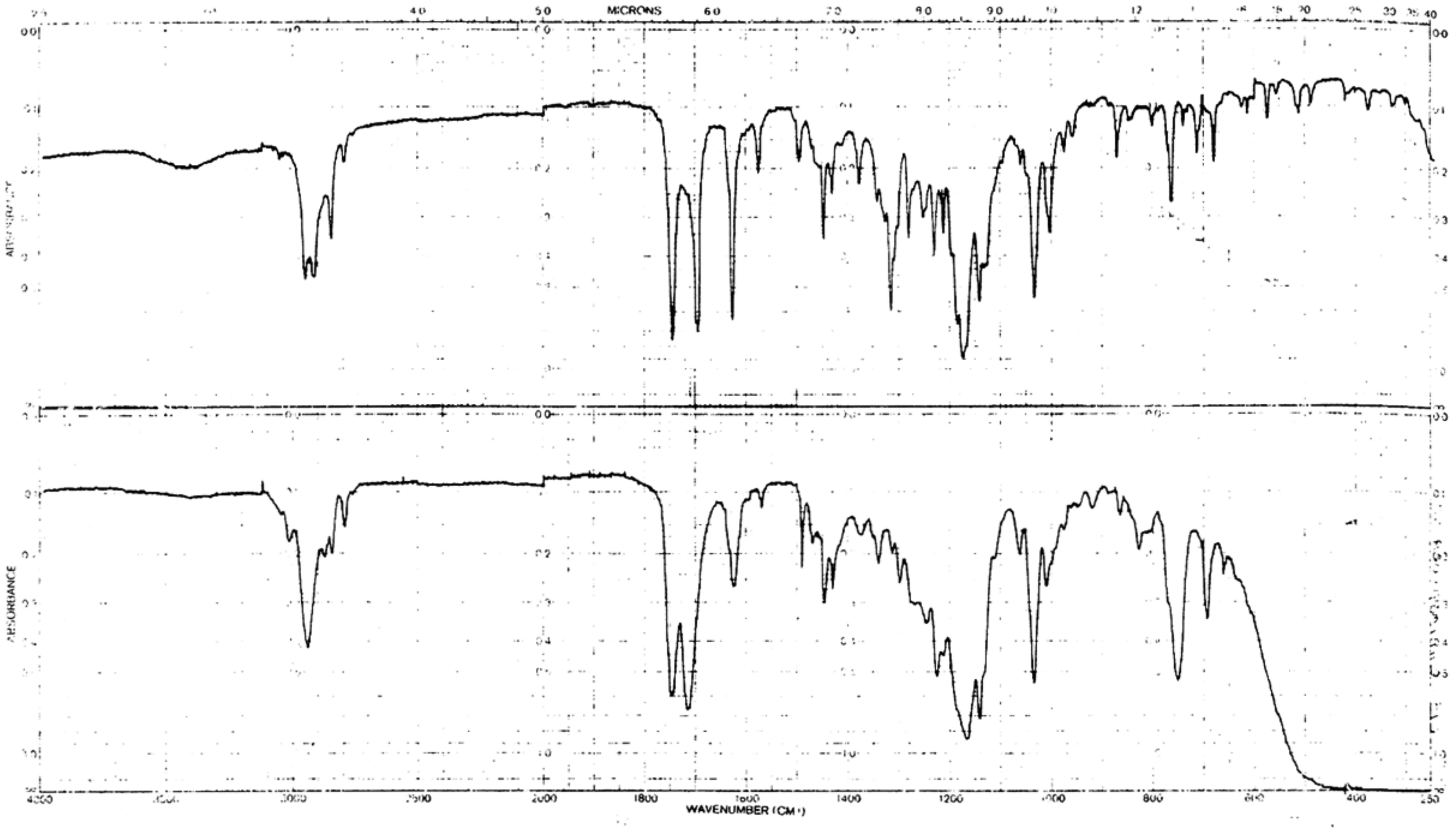

FIG. 6—Infrared spectra of *trans*- and *cis*-cinnamoylcocaine bases (top and bottom, respectively) recorded as a KBr disk and between salt plates, respectively.

bands at the lower frequency differed by about 20 cm^{-1}, with the *trans* isomer absorbing at 1695 cm^{-1}. This difference in frequency between the 2 compounds could also be assigned to differences around the olefinic bond. Apparently, the olefinic proton alpha to the carbonyl group in the *trans* isomer hydrogen bonds with the carbonyl function, causing a decrease in the carbonyl absorption frequency.

A major band present in the *trans* isomer but absent in compound (C) occurred at 1320 cm^{-1}. This absorption band was probably due to the in-plane C—H bending of the olefinic protons in the *trans* isomer.

Another band characteristic of transoid olefins, but absent in *trans*-cinnamoylcocaine, occurs between 960 and 980 cm^{-1} and is caused by out-of-plane bending of the olefinic protons. This band, for example, is prominent in *trans*-cinnamic acid. Its absence in *trans*-cinnamoylcocaine is probably due to the influence of the adjacent carbonyl group. The out-of-plane bending of the olefinic protons may occur at 1000 cm^{-1} where the *trans* isomer exhibits a medium absorption band while compound (C) absorbs weakly.

Infrared spectroscopy confirmed compound (B) as *trans*-cinnamoylcocaine and provided data which suggested that compound (C) was related to (B) in an isomeric manner about the olefinic bond. However, in order to confirm compound (C) absolutely as *cis*-cinnamoylcocaine, it was necessary to subject it to NMR analysis.

Nuclear Magnetic Resonance Analysis

The NMR spectra of compounds (B) (*trans*-cinnamoylcocaine) and (C) were very similar, with most notable differences occurring between 6 and 8 ppm, the range for olefinic proton absorption. Compound (B) produced doublets at 6.25 and 6.50 ppm and at 7.50 and 7.75 ppm. Compound (C) exhibited doublets at 5.80 and 6.00 ppm and at 6.80 and 7.00 ppm. The obvious differences between the 2 compounds are in the chemical shifts of the olefinic protons and in the coupling constants. The smaller chemical shift of the olefinic protons of compound (C) and its smaller coupling constant is characteristic of cisoid behavior when dealing with olefins (8). The identity of compound (C) is thus confirmed as *cis*-cinnamoylcocaine.

Gas Chromatographic Comparison of Identified Cinnamoylcocaines and Illicit Cocaine Seizures

The retention times of *cis*- and *trans*-cinnamoylcocaine were compared with peaks found in re-

fined illicit cocaine samples as well as an illicit sample of Peruvian coca paste (Fig. 3). The paste is an intermediate in the clandestine manufacture of cocaine and contains about 75% cocaine free base; the remainder of the paste consisted of inorganic salts as well as suspected cinnamoylcocaine and tropacocaine. The retention times were the same in every case. Since every illicit cocaine sample cannot be analyzed by combined spectroscopic techniques, GLC identification, using multiple columns in conjunction with other tests already mentioned, serves as adequate identification for the cinnamoylcocaines. Methodology describing the GLC identification of *cis*- and *trans*-cinnamoylcocaine, as well as other contaminants, is the subject of a future paper.

It has been mentioned earlier that detection of cocaine contaminants is essential for the development of meaningful quantitative methodology for cocaine. It has also been suggested that the ability to determine these substances in cocaine seizures can assist enforcement officials in gaining knowledge pertaining to clandestine cocaine laboratories and aid in tracing domestic and international cocaine routes. Finally, it could be of value in narcotic conspiracy cases.

When identifying alkaloids one must always consider the possible formation of artifacts. Cinnamoylcocaine has been established as a bona fide compound in the coca leaf. However, the possibility that either the *cis* or *trans* isomer of cinnamoylcocaine in illicit cocaine seizures is an artifact must be considered. Though a detailed discussion is not the purpose of this paper, a few observations will be made. The possibility that the coca leaf produces the *trans* isomer exclusively and that the *cis* isomer is formed as an artifact during the manufacturing process is not likely. The *trans* isomer is quite stable and none of the *cis* isomer was detected in the synthesized *trans*-cinnamoylcocaine. The other possibilities are that the plant produces only the *cis* isomer with the *trans* being formed as an artifact, or that both isomers are produced with some *trans* being produced as an artifact, or that both isomers are produced by the plant with no artifact formation during cocaine manufacture. In all of the samples examined to date which contained cinnamoylcocaine both isomers were present, usually in roughly equal quantities. A cursory analysis of Bolivian coca leaves showed the presence of both isomers. Further work is planned to determine the significance, if any, of the ratios of the 2 isomers in illicit seizures.

Acknowledgments

I would like to acknowledge the work of Robert P. Barron and Theodore C. Kram for their interpretation of mass spectral and nuclear magnetic resonance data, respectively. My appreciation is also extended to Donald Francis of Stepan Chemical Division for supplying the sample of crude coca bi-alkaloid.

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