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Methyl Esters of Ecgonine: Injection-Port Produced Artifacts from Cocaine Base (Crack) Exhibits

REFERENCE: Casale, J. F., **"Methyl Esters of Ecgonine: Injection-Port Produced Artifacts from Cocaine Base (Crack) Exhibits,"** *Journal of Forensic Sciences*, JFSCA, Vol. 37, No. 5, September 1992, pp. 1295–1310.

ABSTRACT: Many cocaine base (crack) exhibits contain traces of sodium bicarbonate. Pseudoecgonine methyl ester and ecgonine methyl ester are formed as artifacts from the effect of sodium bicarbonate and methanol in the injection port of a gas chromatograph. Formation of ecgonine methyl esters was confirmed by comparison to known standards via gas chromatography/mass spectrometry (GC/MS). The mechanisms for artifact formation are hydrolysis of cocaine, followed by esterification and trans-esterification/base catalyzed epimerization.

KEYWORDS: toxicology, gas chromatography/mass spectrometry, cocaine, crack, ecgonine

Cocaine (Fig. 1, top left) is a naturally occurring alkaloid produced by the plant *Erythroxylum coca*, which is principally grown in the South American countries of Columbia, Peru, and Bolivia. Crack cocaine (cocaine base) is usually made from cocaine hydrochloride in a clandestine setting for the purpose of producing a smokable form of the drug. Cocaine base has a much lower melting temperature than the hydrochloride salt (ca. 98°C compared to 195°C) and readily vaporizes/sublimes at temperatures above 90°C [*1*]. The abuse of cocaine, especially cocaine base ("crack cocaine"), in the United States has grown to epidemic proportions throughout all segments of society.

The identification of cocaine is usually accomplished from infrared or gas chromatography/mass spectrometry (GC/MS) or both analyses. Artifacts that are produced by gas chromatography or sample preparation are a possibility that must always be considered when identifying natural products [*2*]. Lukaszewski and Jeffery [*3*] have reported anhydroecgonine methyl ester (Fig. 1, top right) as a GC injection port produced artifact in cocaine samples. Cocaine base samples that contain sodium bicarbonate have now been found to produce pseudoecgonine methyl ester (Fig. 1, bottom left and ecgonine methyl ester (Fig. 1, bottom right) as injection port artifacts.

Experimental

Mass spectra were obtained on a Hewlett Packard Model 5970 Mass Selective Detector (MSD). A 30-m fused silica DB-5 capillary column (i.d. 0.25mm) (J & W Scientific) at a film thickness of 0.25 μm was used with helium (99.999% UHP) as the carrier gas.

Received for publication 16 Nov. 1991; revised manuscript received 11 Jan. 1992; accepted for publication 14 Jan. 1992.

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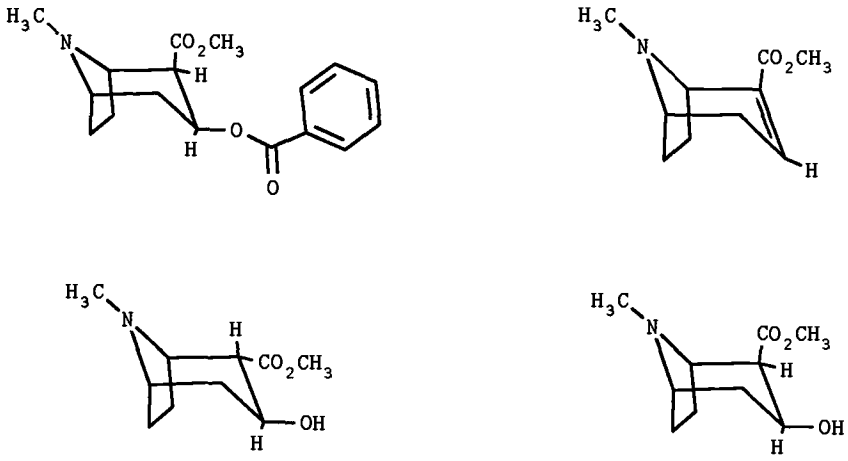

FIG. 1—*Compound structures.*

The injection port temperature was 250°C, and the sample was injected in the split mode (20:1). The initial column temperature was 100°C and was ramped at 10°C/min to 280°C. The quadruple mass analyzer operated under electron ionization conditions at 70 eV and was in full scan mode.

A Hewlett Packard Model 5890 gas chromatograph was used to generate the sample chromatogram via flame ionization detection (FID). Conditions previously reported [*2*] were used.

All reagents and solvents were products of Aldrich Chemical and were used without further purification. Standards of pseudoecgonine methyl ester, ecgonine methyl ester, and anhydroecgonine methyl ester were from an authentic reference collection of this laboratory. An illicit cocaine exhibit containing traces of sodium bicarbonate was obtained and separated into four samples.

The first cocaine sample was derivatized for gas chromatography to determine true alkaloid content [*2*]. In a second experiment, the sample was washed with diethyl ether and filtered for mass spectrometry. In a third experiment, the sample was dissolved in unlabeled methanol for mass spectrometry. Finally, the fourth sample was dissolved in methanol-d_4 for mass spectrometry.

Results and Discussion

Artifacts produced by gas chromatography or sample preparation are a possibility that must always be considered when identifying natural products. Extractions or solvents may inadvertently promote certain artifacts because of their physical and chemical properties. Some known examples of solvents promoting artifact formation are: 1) N-demethylation of cocaine to norcocaine in peroxide enriched diethyl ether, 2) methylation of carboxylic acids in methanol, and 3) epimerization or transesterification or both of esters in alcohol. It is well known that ecgonine methyl ester (Fig. 1, bottom right) is readily epimerized to pseudoecgonine methyl ester (Fig. 1, bottom left) under basic conditions. Specifically, Findlay [*4*] and Siegel [*5*] have shown that the C-2 equatorial epimer is easily formed upon the saponification of cocaine (Fig. 1, top left) by strong base. It has also been shown by Fodor and Kovacs [*6,7*] that ecgonine was irreversibly converted to pseudoecgonine and DeJong [*8,9*] reported that pseudoecgonine could be obtained from ecgonine, ecgonine methyl ester and cocaine. Based on their attempt to interconvert the C-2 axial/equatorial isomers in the related 3-beta-phenyltropane-2-car-

boxylate derivatives Clarke and coworkers [*10*] suggested that the C-2 equatorial (pseudo) configuration was more stable. In fact, Casale and coworkers [*11*] have shown experimentally that pseudoecgonine methyl ester is kinetically and thermodynamically favored over ecgonine methyl ester. Since epimerization involves inversion of configuration at the position alpha to the C-2 position it undoubtedly proceeds by formation of an anion at C-2 followed by reprotonation in the injection port.

A portion of an illicit cocaine base exhibit containing sodium bicarbonate was first subjected to gas chromatography-flame ionization detection (GC-FID) to determine true alkaloid content. Direct derivatization of the sample with N,O-bis(trimethylsilyl)acetamide for GC-FID prohibits artifact formation [*2*]. The sample was shown to contain a small amount of ecgonine, but ecgonine methyl ester, pseudoecgonine methyl ester, and anhydroecgonine methyl ester were not detected (Fig. 2).

Washing the cocaine exhibit with diethyl ether and filtering to remove traces of sodium bicarbonate prior to gas chromatography/mass spectrometry (GC/MS) did produce a small amount of anhydroecgonine methyl ester (Fig. 3). However, ecgonine methyl ester and pseudoecgonine methyl ester were not an effect of diethyl ether as a solvent. These results are consistent with the findings of Lukaszewski and Jeffery. Artifact formation of anhydroecgonine methyl ester undoubtably is the result of elimination of benzoic acid from cocaine in the heated injection port of the GC/MS.

When the sample containing traces of sodium bicarbonate was dissolved in unlabeled

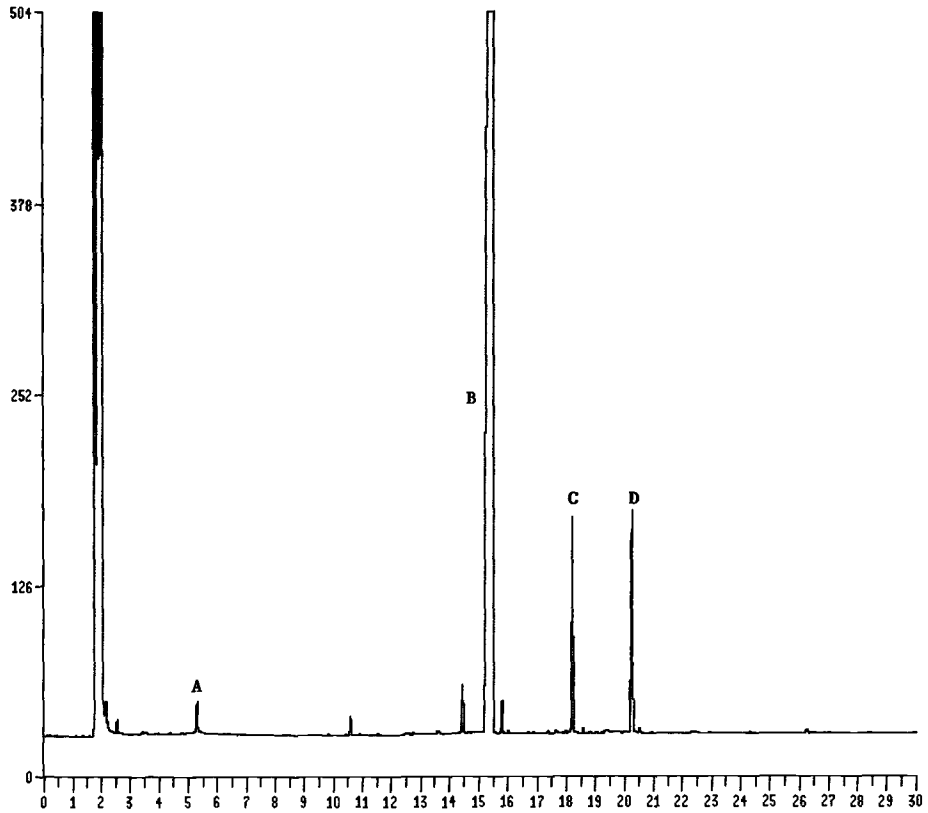

FIG. 2—*Derivatized capillary GC-FID of cocaine base sample. A = ecgonine, B = cocaine, C = cis-cinnamoylcocaine, D = trans-cinnamoylcocaine.*

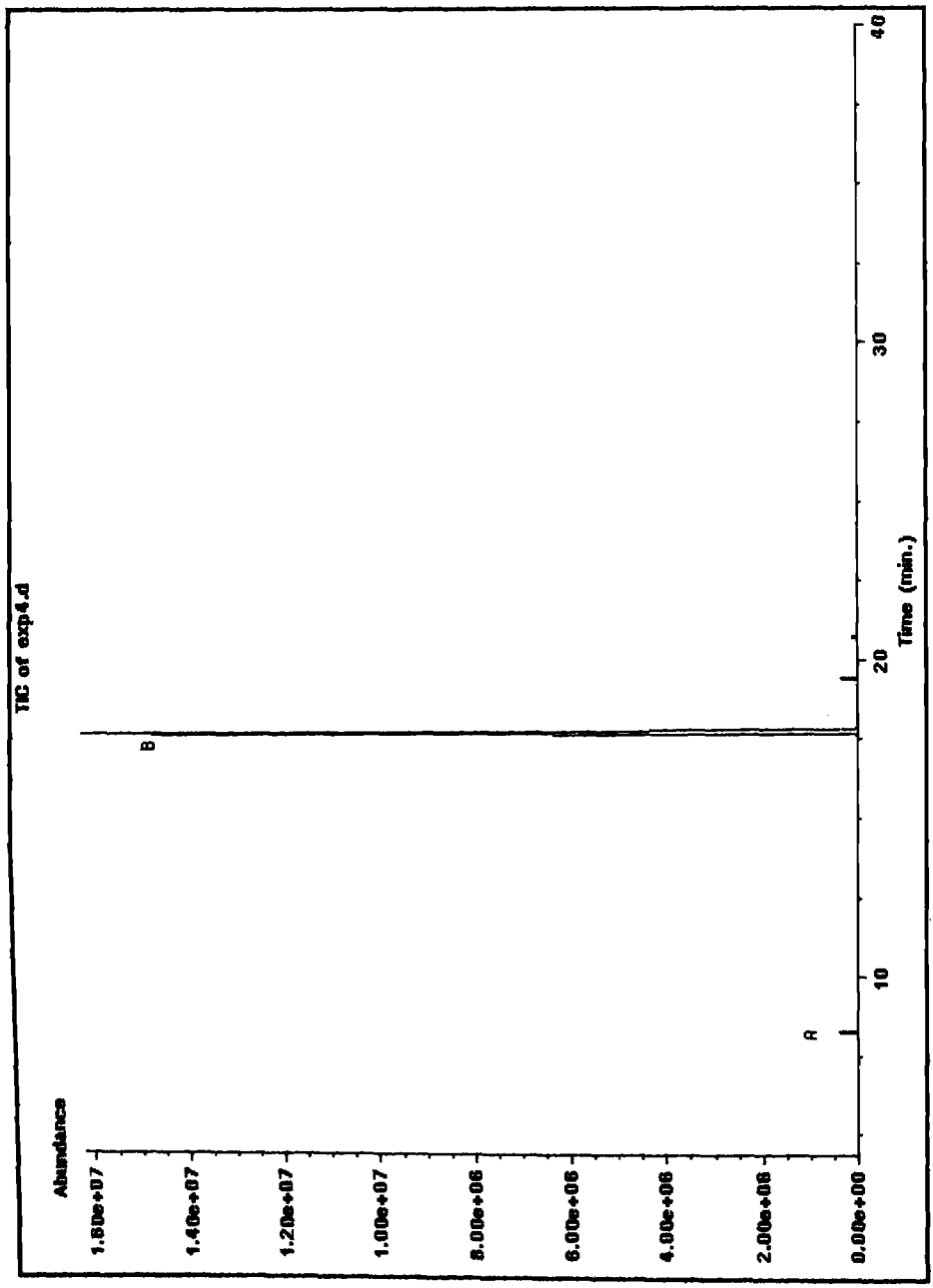

FIG. 3—*Reconstructed ion chromatogram of cocaine base sample using diethyl ether as solvent. A = anhydroecgonine methyl ester, B = cocaine.*

methanol and subjected to GC/MS, three artifacts were produced (Fig. 4). Artifacts of anhydroecgonine methyl ester, ecgonine methyl ester, and pseudoecgonine methyl ester, were identified by comparing their respective mass spectra (Figs. 5, 6, and 7) to known standards. The relative amount of anhydroecgonine methyl ester is much more pronounced as compared to the diethyl ether injection. The formation of ecgonine methyl ester and pseudoecgonine methyl ester as artifacts proceeds through a separate mechanism from anhydroecgonine methyl ester. In this instance, hydrolysis of cocaine occurs in the heated injection port to yield ecgonine or ecgonine methyl ester, or both followed by esterification and transesterification. The esterification is catalyzed and enhanced with the presence of traces of bicarbonate. The combination of bicarbonate and methanol at elevated temperatures will yield sodium methoxide, a very effective basic methylating agent. Sodium methoxide is also quite effective in extracting the weakly acidic proton at C-2 for anion formation. Once the C-2 anion is formed, reprotonation at C-2 can proceed through either the alpha- or beta-face to give ecgonine methyl ester and pseudoecgonine methyl ester respectively.

Substituting methanol-d_4 for (sample containing traces of sodium bicarbonate) introduction to the GC/MS also led to the three artifacts (Fig. 8) in roughly the same concentrations but produced substantially different mass spectra for anhydroecgonine methyl ester, ecgonine methyl ester, and pseudoecgonine methyl ester (Figs. 9, 10, and 11). Anhydroecgonine methyl ester (Fig. 9) has become incorporated with three deuteriums at marginally 70% d_3-isotopic purity. Incorporation was verified at the methoxy methyl position by shift of three Daltons at the m/z 152 base peak and the m/z 181 molecular ion. The 3-carbomethoxy-N-methylpyridinium cation (m/z 152) shifted substantially to m/z 155, which is consistent with transesterification of the methoxy methyl in methanol-d_4. It should be noted that the proton at C-3 of anhydroecgonine methyl ester remained intact (no exchange or incorporation, that is, an increase of 4 Daltons needed), thus supporting the mechanism of thermal elimination of benzoic acid from cocaine as proposed by Lukaszewski and Jeffery [*3*].

Ecgonine methyl ester (Fig. 10) is incorporated with deuterium as a mixture of isotopomers (d_0, d_1, d_3, d_4, and d_5). The N-methylpyrrolidinium cation m/z 82, representing the base peak for ecgonine methyl ester was unaffected, therefore, if deuterium incorporation had taken place within the five membered ring, a shift of one or more Daltons would be expected for this ion. Since there was no change in the relative intensities of the ions of m/z 82 and 83 compared to the unlabeled ester (Fig. 6), it is clear that none of these positions had become deuterated. The N-methylpyridinium cation (m/z 94), representing the six-membered ring fragment of the bicyclic system, had a partial shift of one Dalton (ca. 30% of m/z 94). This cation retains the proton at its 3 position by losing the hydroxyl and carbomethoxy groups, thus giving rise to a m/z 95 ion with a substantial collapse in m/z 94. By deduction it then follows that some deuterium incorporation had taken place at C-2 through anion formation prior to electron ionization in the source of the mass spectrometer. A small shift in the cation m/z 96 was observed and is most likely due to exchange at C-4 through C-3,4 enol-keto tautomerism. Ion m/z 202 represents the molecular ion of the isotopomer containing a fully incorporated methoxy methyl-d_3 and is representative of transesterification of ecgonine methyl ester or esterification of ecgonine with methanol-d_4. Ion m/z 203 also depicts the deuterated methoxy methyl with a combination of deuterium exchange at C-2 or the hydroxyl proton at C-3. The molecular ion for the d_5-isotopomer (m/z 204) represents incorporation at the methoxy methyl, C-2 proton, and the hydroxyl proton. The shift of the unlabeled molecular ion (m/z 199) by one Dalton to m/z 200 is analogous to hydroxyl exchange as seen in ions m/z 202 and m/z 203.

Pseudoecgonine methyl ester (Fig. 11) has incorporated with deuterium as two isotopomers (d_4 and d_5). The N-methylpyrrolidinium cation m/z 82, representing the base

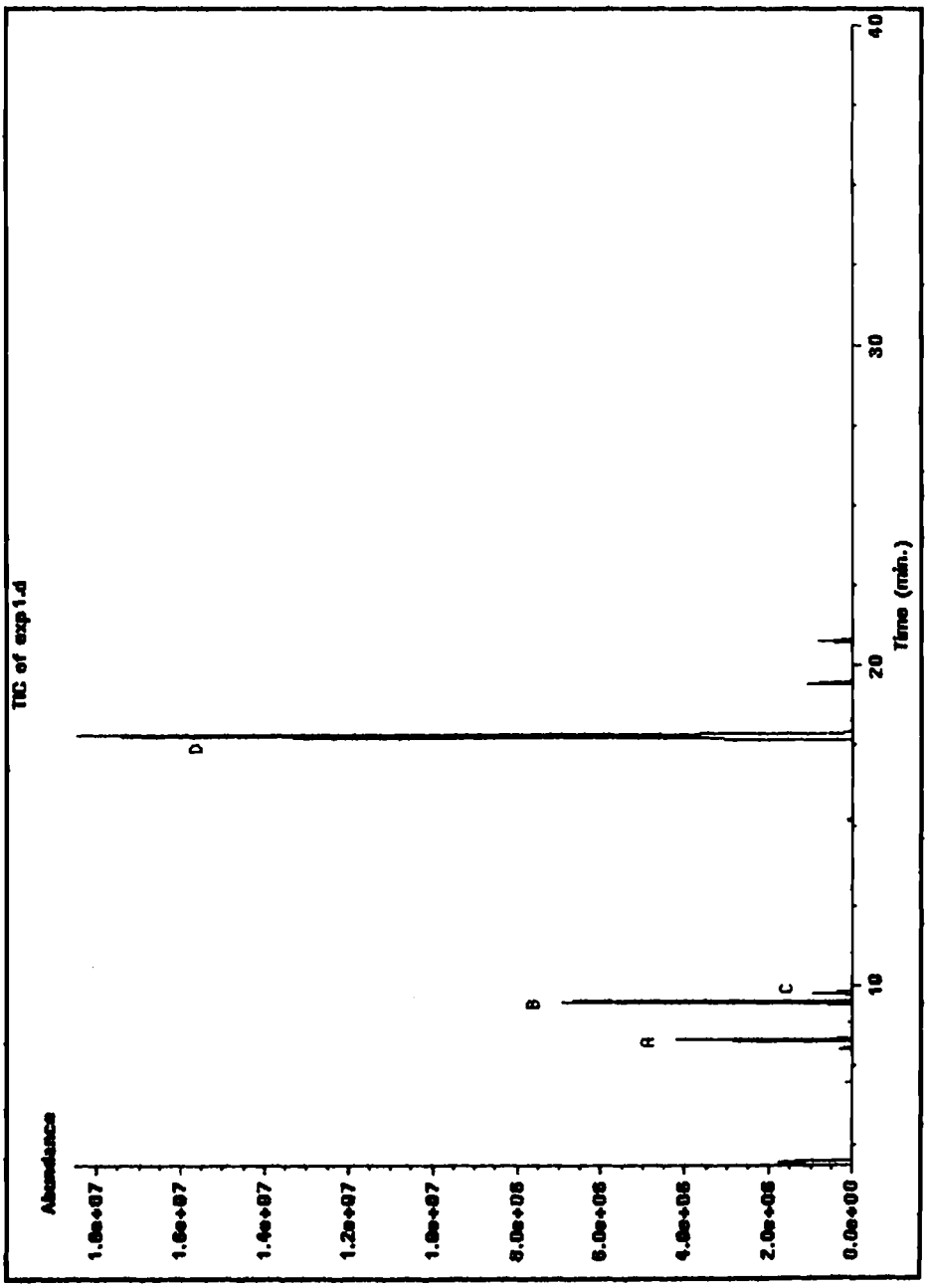

FIG. 4—*Reconstructed ion chromatogram of cocaine base sample using unlabeled methanol as solvent. A = anhydroecgonine methyl ester, B = ecgonine methyl ester, C = pseudoecgonine methyl ester, D = cocaine.*

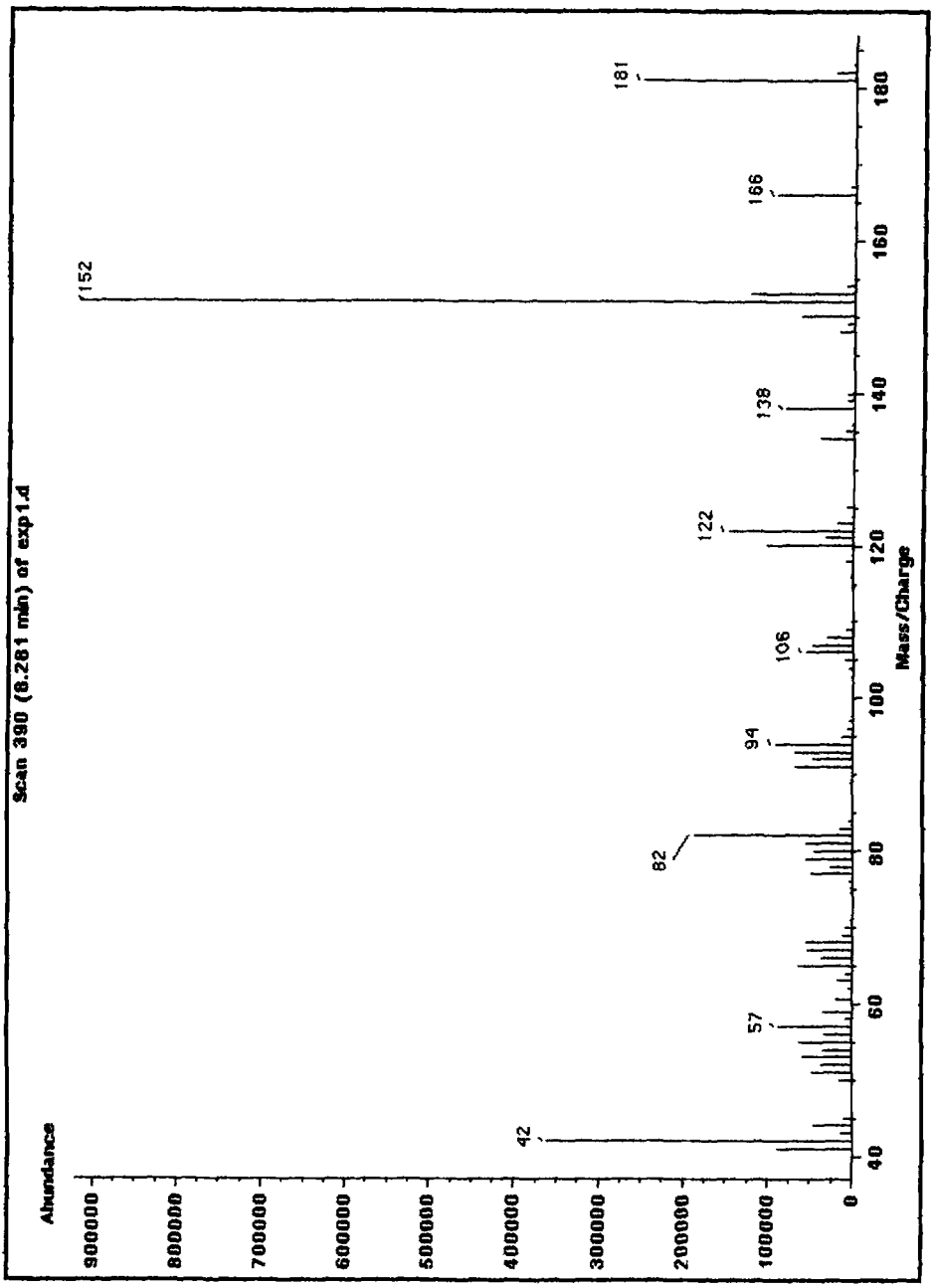

FIG. 5—*Electron ionization mass spectrum of anhydroecgonine methyl ester.*

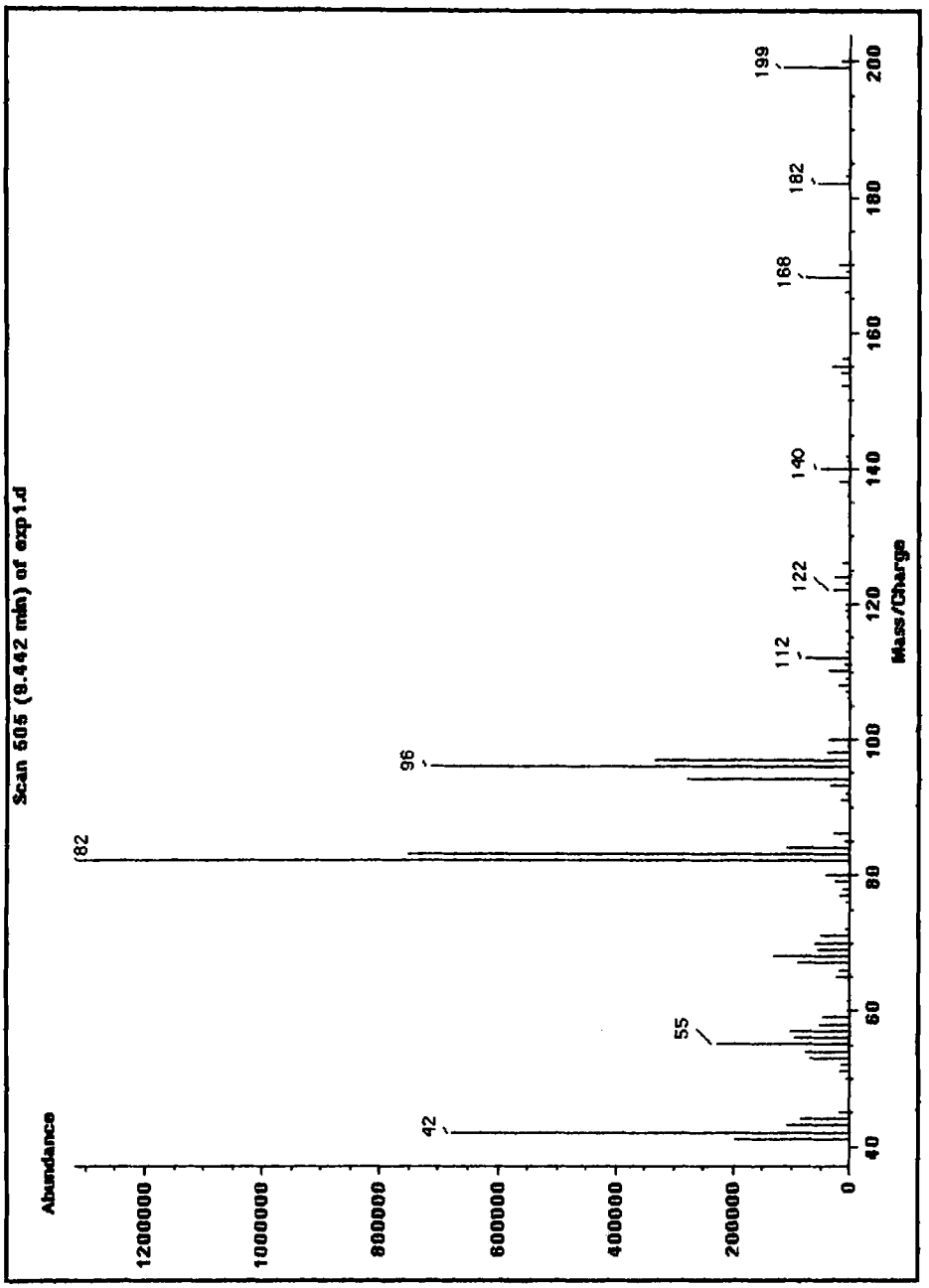

FIG. 6—*Electron ionization mass spectrum of ecgonine methyl ester.*

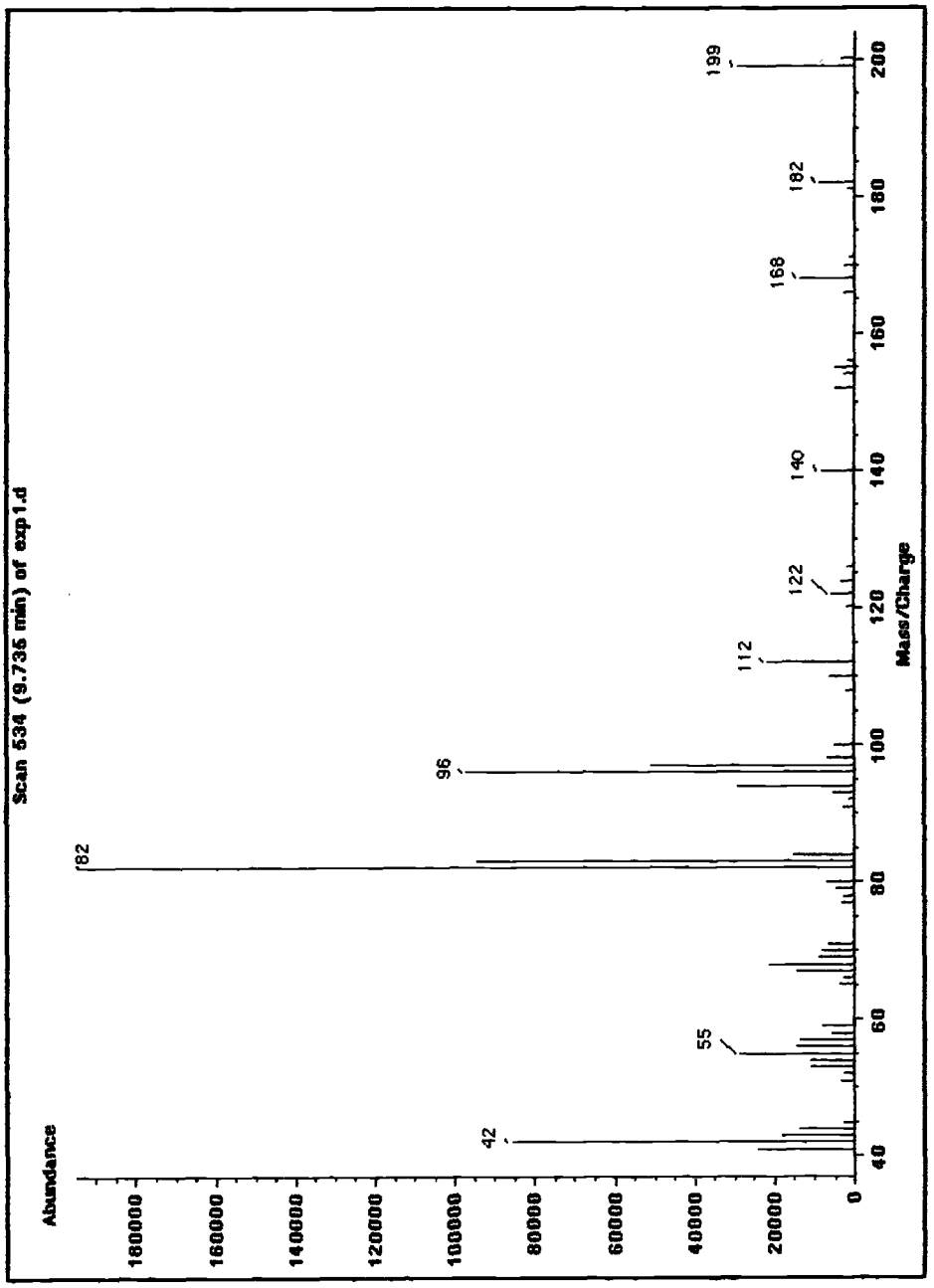

FIG. 7—*Electron ionization mass spectrum of pseudoecgonine methyl ester.*

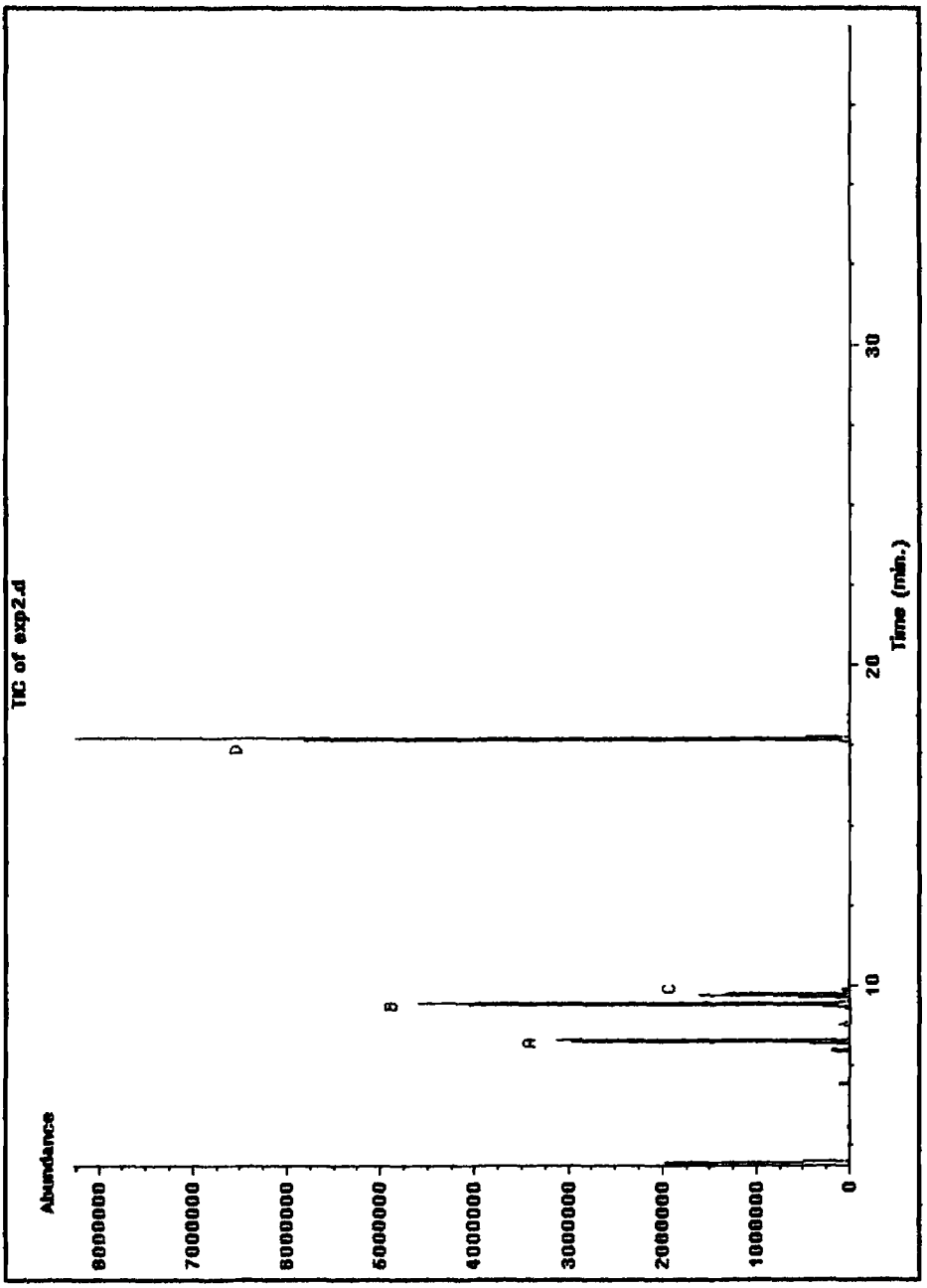

FIG. 8—*Reconstructed ion chromatogram of cocaine base sample using methanol-d_4 as solvent. A = anhydroecgonine methyl ester, B = ecgonine methyl ester, C = pseudoecgonine methyl ester, D = cocaine.*

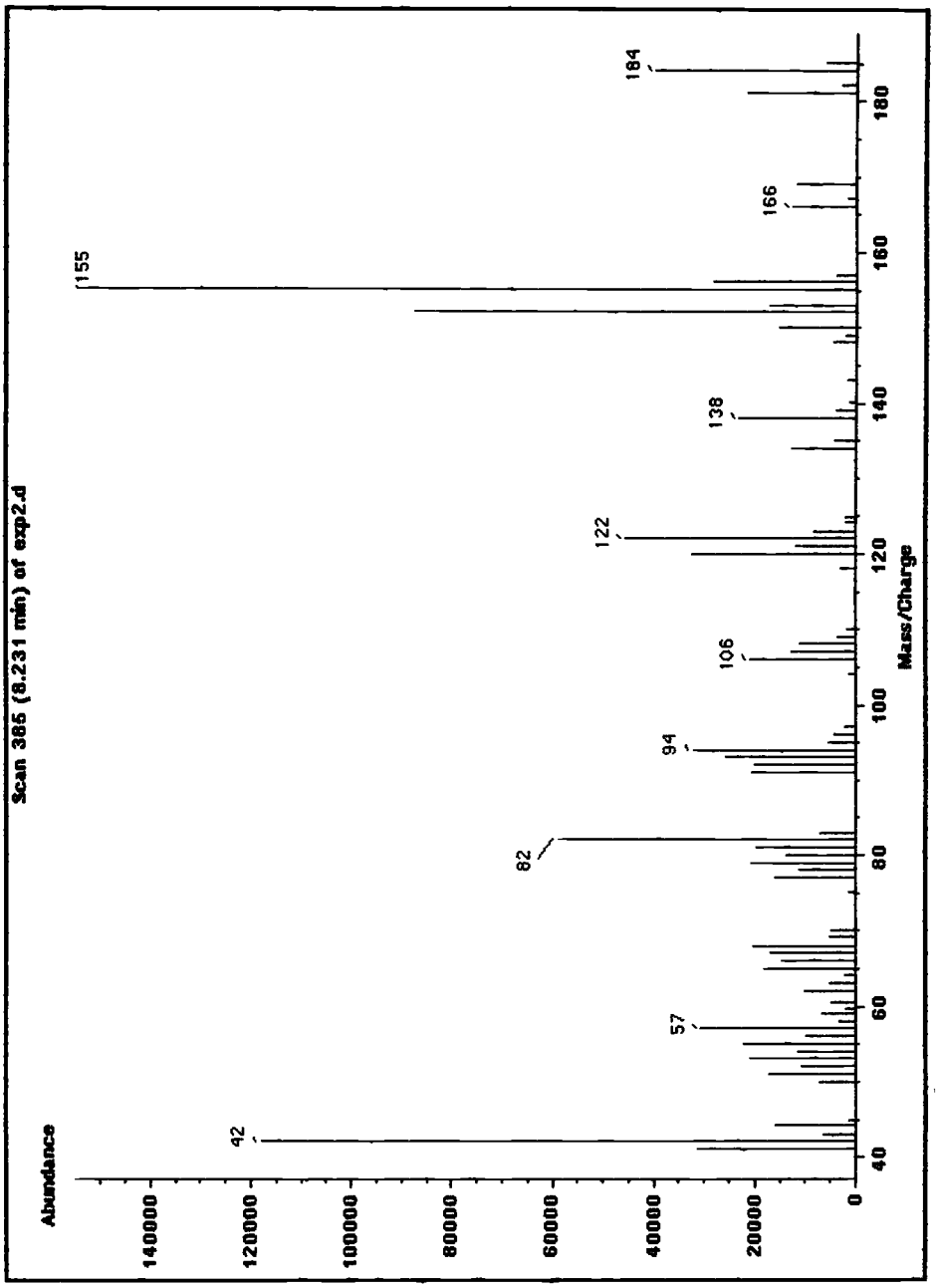

FIG. 9—*Electron ionization mass spectrum of deuterium incorporated anhydroecgonine methyl ester.*

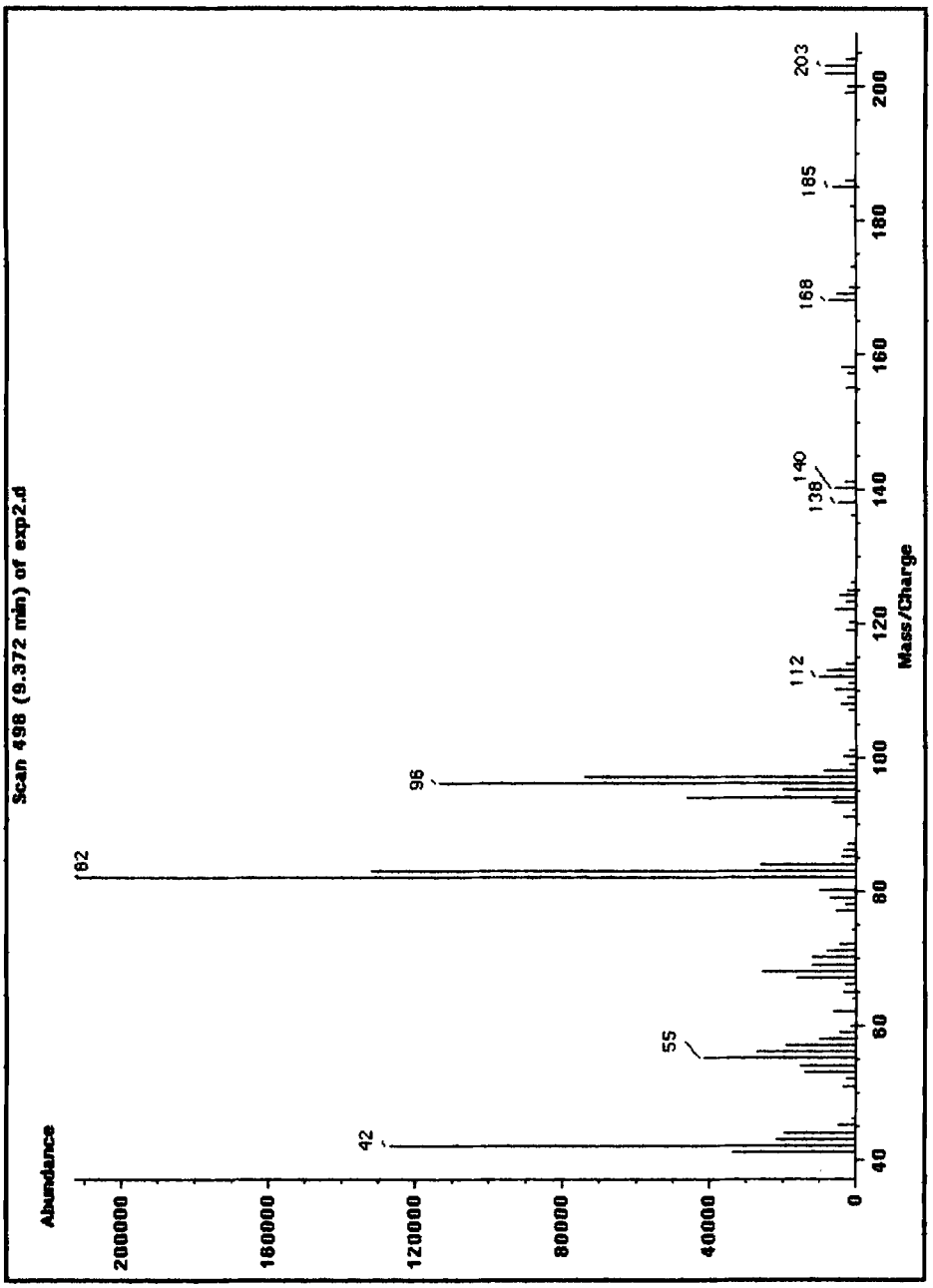

FIG. 10—*Electron ionization mass spectrum of deuterium incorporated ecgonine methyl ester.*

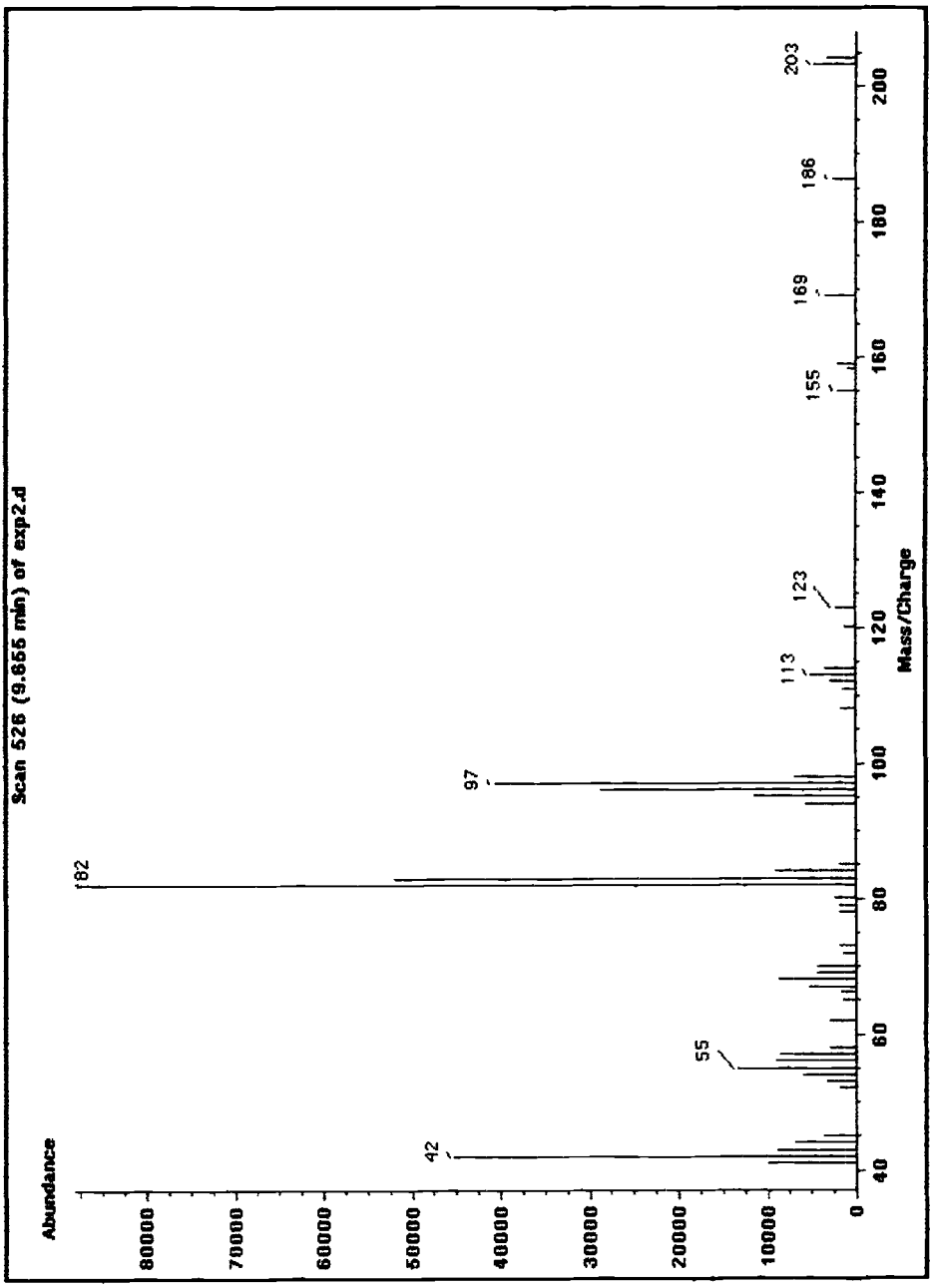

FIG. 11—*Electron ionization mass spectrum of deuterium incorporated pseudoecgonine methyl ester.*

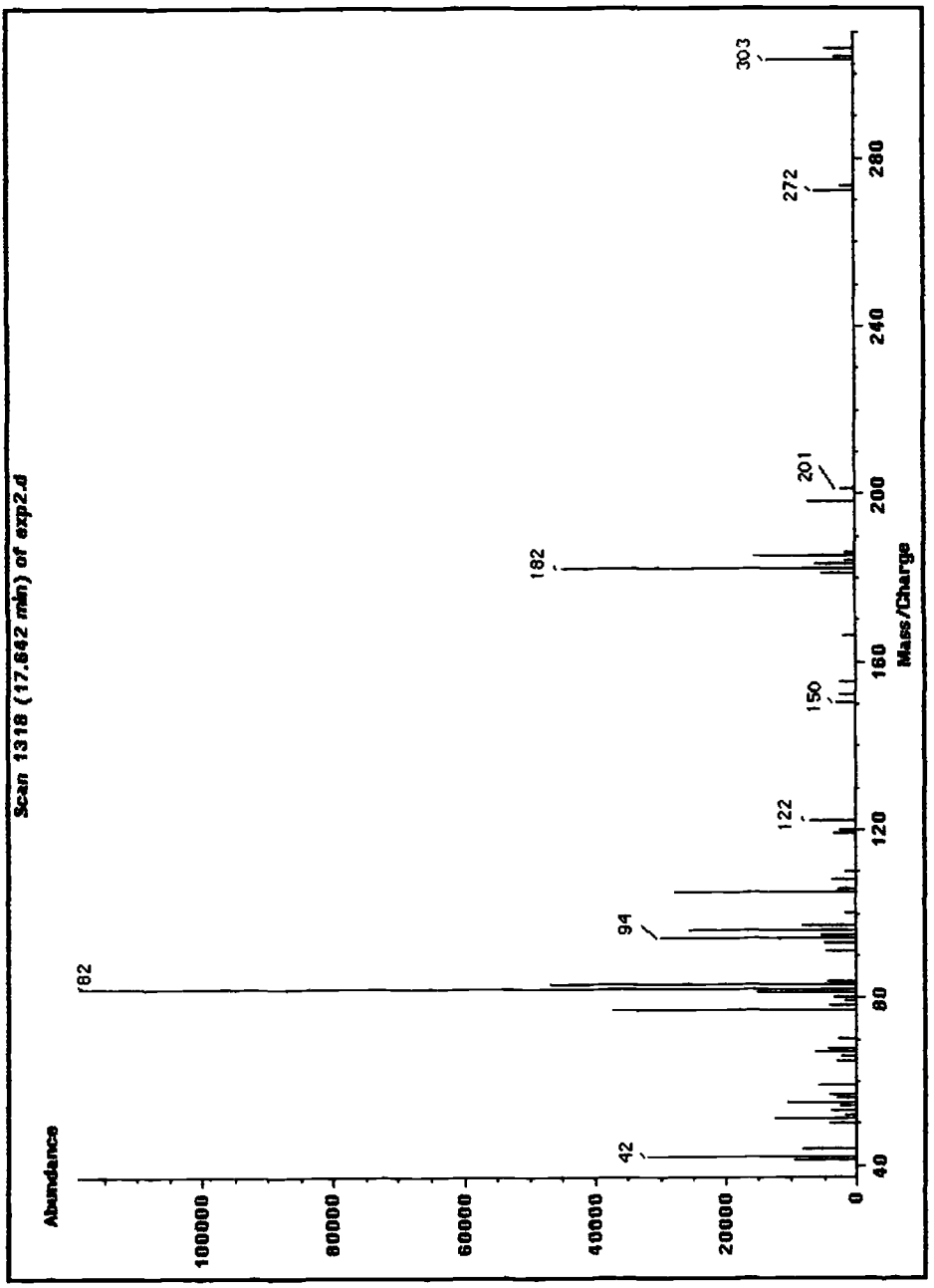

FIG. 12—*Electron ionization mass spectrum of deuterium incorporated cocaine.*

peak for pseudoecgonine methyl ester was unaffected, therefore, as in ecgonine methyl ester, it is clear that incorporation was not within the five-membered ring fragment. The N-methylpyridinium cation (m/z 94) had a substantial shift of one Dalton (ca. 65% of m/z 94). Therefore deuterium incorporation had taken place at C-2 via the same mechanism as ecgonine methyl ester. Cation m/z 96 also shifted substantially more to m/z 97 than in ecgonine methyl ester. Ion m/z 203 represents the molecular ion of the d_4-isotopomer containing a fully incorporated methoxy methyl-d_3 and deuterium at C-2. The mechanism is transesterification and epimerization of ecgonine methyl ester with methanol-d_4. The molecular ion for the d_5-isotopomer (m/z 204) depicts incorporation at the methoxy methyl, C-2 proton, and the hydroxyl proton which is analogous to the d_5-isotopomer of ecgonine methyl ester.

Evidence for transesterification of cocaine was observed on the leading edge of the eluting cocaine peak. A partial shift of three Daltons of the molecular ion m/z 303 to m/z 306 was observed (Fig. 12). If epimerization to pseudococaine had occurred, the proton at C-2 would be exchanged and a shift of four Daltons would have been expected. The spectra obtained is most probably the coelution of unlabeled cocaine and cocaine incorporated with a methoxy methyl-d_3.

Summary

The formation of ecgonine methyl ester and pseudoecgonine methyl ester as artifacts in gas chromatographic/mass spectral analysis of illicit cocaine base exhibits containing traces of sodium bicarbonate is discussed. Using deuterium-labeled methanol, the mechanisms for artifact formation were determined to be hydrolysis of cocaine, followed by esterification and transesterification/base catalyzed epimerization. The formation of anhydroecgonine methyl ester as an artifact via thermal elimination of benzoic acid from cocaine is also supported from the experimental data.

Acknowledgments

This work was supported in part by Grant #170-188-E6-D009 and Grant #170-190-E6-D034 of the U.S. Department of Justice.

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