Journal of Analytical Toxicology, Vol. 14, November/December 1990

# A Pyrolysis Product, Anhydroecgonine Methyl Ester (Methylecgonidine), is in the Urine of Cocaine Smokers

**Peyton Jacob, III,* Evan R. Lewis, Barbara A. Elias-Baker,** and **Reese T. Jones**
*Drug Dependence Research Center, Langley Porter Psychiatric Institute, University of California-San Francisco, San Francisco, California 94143*

## Abstract

**A method using combined gas chromatography/mass spectrometry (GC/MS) for determination of the cocaine pyrolysis product anhydroecgonine methyl ester (AEME) in urine is described. Using this method, we found that human subjects who smoked cocaine under laboratory conditions excreted substantial amounts of AEME in their urine. Little, if any, AEME was excreted in the urine when the same subjects were administered cocaine by intravenous and intranasal routes. AEME may be a useful marker for cocaine (crack) smoking. The pharmacology of AEME is unknown. The possibility that AEME may play a role in the effects associated with cocaine smoking needs to be examined.**

## Introduction

Abuse of "crack," a free-base cocaine that is smoked by converting it to an aerosol and inhaling it from a heated pipe, or in a mixture of marijuana or tobacco in cigarettes, is a serious social and health problem. Heating cocaine under conditions that mimic smoking produces various decomposition products (1-3), including benzoic acid, methyl benzoate, *N*-methylbenzamide, methyl cycloheptatrienecarboxylate isomers, methyl-4-(3-pyridyl)-butyrate, and isomers of the product resulting from the elimination of benzoic acid from cocaine (anhydroecgonine methyl ester and isomeric compounds). To what extent these substances are absorbed by cocaine smokers and whether they contribute to the pharmacology or toxicology of cocaine smoking is unknown.

A recent study (3) and studies in our laboratory indicate that anhydroecgonine methyl ester (AEME, methylecgonidine) is the major volatile pyrolysis product formed under conditions similar to free-base cocaine smoking. Consequently, we thought it important to determine if AEME is absorbed by cocaine smokers. In this report, we describe a method for determination of AEME in urine and report data on urinary excretion of AEME in cocaine smokers.

* Address correspondence to: Peyton Jacob, III, San Francisco General Hospital, Building 100, Room 235, 1001 Potrero Avenue, San Francisco, California 94110.

## Materials and Methods

*Chemicals and reagents.* The anhydroecgonine methyl ester (AEME) standard was prepared by the method of Zirkle (4) and converted to the crystalline picrate salt, mp 213.5-214.5°C. The mass spectrum was in good agreement with a published spectrum (5). The internal standard, anhydroecgonine ethyl ester (AEEE), was prepared from anhydroecgonine (4) by the method of Findlay (6) and converted to the picrate, mp 167.5-168.5°C. Methyl-t-butylether and toluene were HPLC grade from Aldrich Chemical and 1-butanol was HPLC grade from Fisher Scientific. Aqueous potassium carbonate and sulfuric acid solutions were made from distilled water and reagent grade chemicals. The 5% palladium on barium sulfate, reduced, and sodium borohydride, 99%, were obtained from Aldrich. Cocaine hydrochloride administered to the subjects was purchased from Mallinckrodt and was of USP grade. Cocaine base was prepared by treating the hydrochloride with aqueous potassium carbonate.

*Subjects.* Fifteen healthy, paid volunteer subjects (eight men and seven women) who were experienced cocaine users, 20-33 years of age, were admitted to the General Clinical Research Center at San Francisco General Hospital Medical Center one day before cocaine administration. All had experience with cocaine smoking. Body weight ranged from 55 to 98 kg with a mean of 65 kg. Informed consent was obtained after the nature and possible consequences of the studies had been fully explained. The protocol was approved by the University of California-San Francisco, Committee on Human Research.

*Experimental protocol for cocaine administration.* The smoking experiments used a 1-L 3-neck round-bottom flask connected to a 15-cm length of 8-mm i.d. glass tube, bent in a 45° angle serving as a mouthpiece. The flask was heated to 250°C (surface temperature) with a heating mantle and 100 mg of finely powdered cocaine base was dropped into the flask. Thirty seconds later, the subjects were asked to take two successive deep inhalations of 10-s duration approximately 45 s apart. The nasal dose was 2 mg/kg of body weight and was given as a 20% cocaine hydrochloride in physiologic saline solution by a Forrester sprayer with 4-6 repeated fine sprays over a 30-s period. The subjects vigorously sniffed during each spray. The intravenous dose was cocaine hydrochloride, 0.6 mg/kg of body weight, injected by syringe pump over 1 min into a large forearm vein. Usually, 3-4 days separated experiments. The selection of route of administration was in balanced, modified Latin square

order. Subjects were also given oral cocaine, 2 mg/kg, and placebo for cocaine, but those data are not reported in detail here. Urine samples collected at intervals for 48 h following cocaine administration were immediately acidified to a pH of 2–3 (to stabilize cocaine and AEME) and stored frozen at −20°C.

*Extraction/derivatization procedure.* A flow diagram of the extraction/derivatization procedure is shown in Figure 1. One-mL aliquots of urine or standard, 100 μL of the internal standard, 5 μg/mL aqueous anhydroecgonine ethyl ester, 1 mL methyl-t-butylether–2-propanol (9:1), and 0.5 mL 50% (w/v) aqueous potassium carbonate were pipetted into 16 × 100-mm culture tubes with teflon-lined screw caps. The tubes were capped and vortexed for 3 min on a multitube vortexer (Troemner, Model VX-5000) and centrifuged for 3 min on a desktop clinical centrifuge (Beckman, Model TJ-6) to separate phases. The tubes were cooled in a mixture of dry ice and acetone to freeze the aqueous phases, and the organic phases were decanted into 13 × 100-mm screw-topped culture tubes containing ≈5 mg of 5% palladium on barium sulfate and ≈5 mg sodium borohydride which had been previously mixed by vortexing. The capped tubes were allowed to stand at room temperature for 20 min. To each tube was added 1 mL of 4N aqueous sulfuric acid. The tube was then vortexed 3 min, centrifuged 3 min, the aqueous phases frozen in the dry ice–acetone bath, and the (top) organic phases discarded. After thawing, the aqueous phases were made alkaline by the addition of 0.5 mL 50% (w/v) aqueous potassium carbonate and extracted with 50 μL toluene–butanol (9:1) by vortex mixing for 3 min. The tubes were centrifuged 3 min, cooled in the dry ice–acetone bath, and the organic phases decanted into 300-μL conical microvials. The microvials were crimp-capped with teflon-lined caps, and 3-μL aliquots were injected via autosampler into the GC/MS.

*Gas chromatography/mass spectrometry.* The GC/MS analyses were performed on a Hewlett-Packard Model 5890 GC equipped with a Hewlett-Packard Model 5970B mass selective detector, a Hewlett-Packard Model 7673A autoinjector system, and a Hewlett-Packard Model 59980 MS ChemStation data system. The capillary GC column was a Hewlett-Packard HP-1 (crosslinked methylsilicone gum, 12 m × 1.2 mm i.d. × 0.33-μm film thickness) with a Carbowax-treated, glass injection port liner configured for splitless injection. The linear flow rate of the helium carrier gas was 3 m/min. Both the injection port and MS transfer line were heated to 260°C. Following injection, which was made in the splitless mode, the column temperature was held at 70° for 0.5 min, then ramped at 20°/min to 120°, held at 120° for 5.1 min, then ramped at 70°/min to 275°and held at 275°C for 0.5 min. Ionization was carried out in positive EI mode at 70 eV with the emission current at 220 μA. The mass spectrometer was tuned with PFTBA using the Full Autotune routine of the Hewlett-Packard Model 59980 MS ChemStation software package. Single ion monitoring of *m/z* 183 (4.9–6.2 min) and *m/z* 197 (6.2–8.0 min) was employed to detect the molecular ions of the reduction products of anhydroecgonine methyl ester and anhydroecgonine ethyl ester, respectively. Mass spectra were obtained by scanning *m/z* 40–190.

*Quantitation.* Stock solutions of the picrate salts of anhydroecgonine methyl ester and anhydroecgonine ethyl ester (1 mg/mL of the free base) were prepared in methanol; further dilutions were made with 0.01N aqueous sulfuric acid and blank urine (adjusted to pH 3) for preparation of standards. Six or more aqueous standards were included with each batch of samples and used to construct a standard curve based on peak height ratios of the major epimers of the reduction products of AEME (RT 5.66 min) and the internal standard (RT 7.29 min). Standard curves were linear in the range of 0–5,000 ng/mL. A typical standard curve equation was as follows: response ratio = 0.930 (amount ratio) + 0.0732, $r^2$ = 0.992, in which response ratio = peak height of AEME/peak height of internal standard, and amount ratio = amount of AEME/amount of internal standard. Control samples, prepared by spiking blank urine, adjusted to pH = 3, at three concentrations spanning the expected concentration range, were included in each batch of unknown samples.

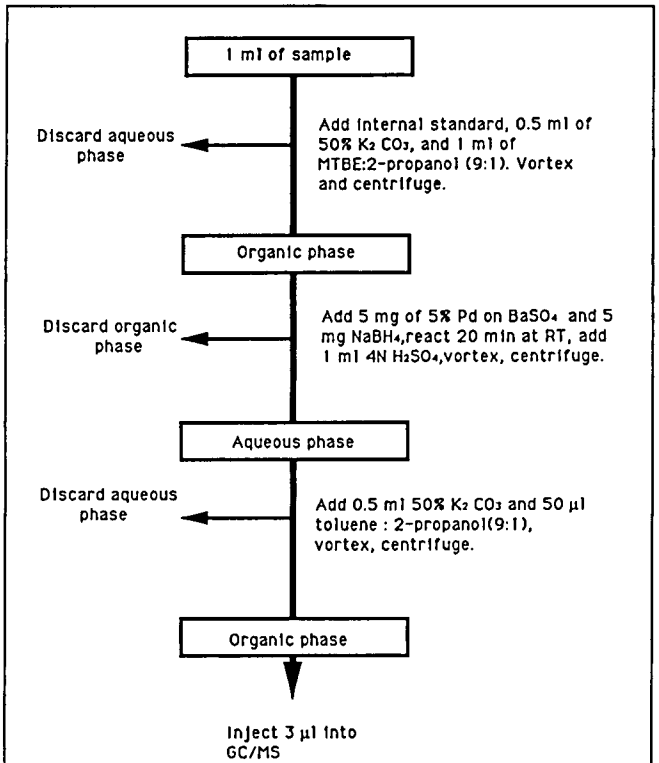


**Figure 1.** Flow diagram of extraction and derivatization procedure.

## Results

Preliminary studies indicated that AEME (Structure 2, Figure 2) is a major pyrolysis product of cocaine (Structure 1, Figure 2) under a variety of conditions and that it was absorbed by research subjects who smoked cocaine (9). Consequently, we developed a GC/MS method suitable for routine determination of AEME in biological samples.

The procedure for extraction of AEME from urine, derivatization, and analysis by GC/MS is shown in Figure 1. A structural analog, anhydroecgonine ethyl ester (AEEE), is used as an internal standard. The extract is treated with sodium borohydride and a palladium catalyst, which reduces the carbon-carbon double bond of AEME to produce derivative 5 (Figure 2). The derivatization reaction was found to proceed readily on nanogram quantities of AEME extracted from urine. AEEE undergoes an analogous reduction. The reduction products of AEME and the internal standard have good chromatographic properties and were readily separated on a 12-m methylsilicone capillary column (Figure 3). Quantitation was achieved by selected ion monitoring of the molecular ions of reduced AEME and the internal standard, *m/z* 183 and 197, respectively, and

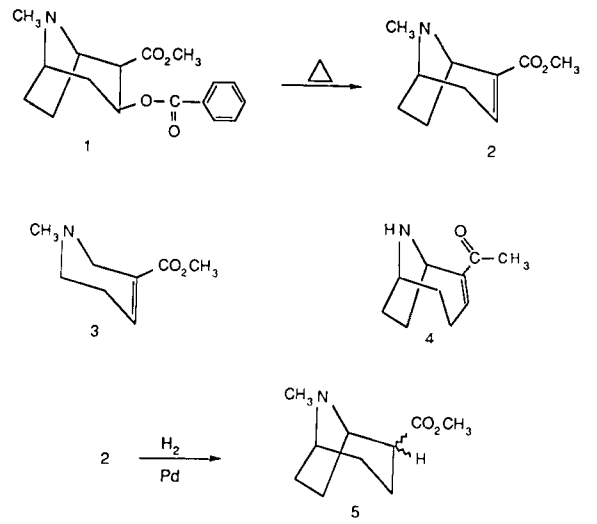


**Figure 2.** Structures of cocaine (1), anhydroecgonine methyl ester (2), arecoline (3), anatoxin (4), and anhydroecgonine methyl ester reduction product (5).

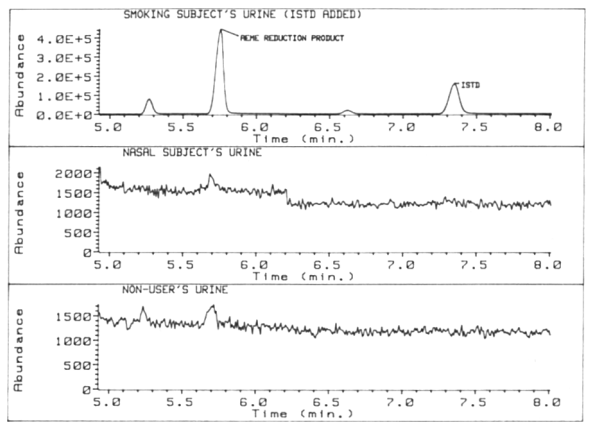


**Figure 3.** Ion chromatograms of urine extracts from a human subject following cocaine smoking (upper panel), from a human subject following intranasal cocaine administration (middle panel), and from a drug free subject (lower panel).

integration of the reconstructed ion chromatograms. Standard curves, based on peak heights, were linear from 50–5,000 ng/mL. The assay has good precision, with coefficients of variation ranging from 3–11% over the concentration range of 50–2,000 ng/mL for spiked urine samples (Table I). Chromatograms of extracts from drug-free urine were free of interfering substances (Figure 3). The sensitivity of the method is excellent, with a signal-to-noise ratio of about three to one at 10 ng/mL.

To determine the extent that AEME is absorbed by cocaine smokers, we analyzed urine samples from human subjects who smoked cocaine. Urine samples were collected at regular intervals for 48 h after cocaine administration and analyzed for cocaine, benzoylecgonine, and AEME. After smoking, most subjects excreted substantial amounts of AEME (Table II), averaging about half the molar amount of cocaine excreted (Table III). Nine subjects excreted a mean of 0.85 μmol of AEME (range 0.09–3.74) during the 48-h collection period. These same subjects excreted a mean of 1.58 μmol of cocaine (range 0.25–4.25) and 21.5 μmol benzoylecgonine (range 6.45–46.7) (Table III). Urinary concentrations of AEME averaged 815.9 ng/mL (range 51–2,866) in urine collected over the first 2 h following cocaine smoking, and then rapidly decreased over the course of the remaining collection periods (Figure 4). Elimination half-life estimated from the urine data indicates that AEME has a relatively short half-life of about 1 h. The identity of AEME in the urine of a cocaine smoker was confirmed by GC/MS analysis with the mass spectrometer operated in the scan mode. The mass spectrum and retention time of the AEME reduction product produced from the cocaine smoker's urine were virtrually identical to those of a reduced standard (Figure 5).

We also analyzed urine samples after cocaine was admini-

**Table I. Intra-Day Precision for Determination of Anhydroecgonine Methyl Ester in Urine**

| No. of analyses | Concentration, ng/mL | | CV (%) |
|---|---|---|---|
| | Added | Measured, mean | |
| 6 | 50 | 56 | 5.4 |
| 8 | 200 | 230 | 7.0 |
| 6 | 500 | 565 | 7.6 |
| 8 | 1000 | 967 | 3.0 |
| 6 | 2000 | 2190 | 11 |

**Table II. Cumulative Urinary Excretion of Anhydroecgonne Methyl Ester (AEME) Following Cocaine Administration**

| Subject | AEME excreted (μmol) 0-48 h | | |
|---|---|---|---|
| | Smoked | Intravenous | Intranasal |
| 87132 | 0.81 | | |
| 88139 | 0.093 | 0 | |
| 88148 | 0.39 | 0 | 0.002 |
| 88153 | 1.09 | 0.034 | 0.036 |
| 88154 | 0.11 | | |
| 88164 | 0.65 | 0.012 | 0 |
| 88170 | 3.74 | 0 | 0.062 |
| 88173 | 0.53 | | 0.14* |
| 88176 | 0.21 | 0 | 0.011 |
| Mean | 0.85 | 0.0077 | 0.042 |

* This subject was suspected of having used illicit cocaine during the period following nasal administration.

**Table III. Mean Urinary Excretion of Anhydroecgonine Methyl Ester (AEME), Cocaine, and Benzoylecgonine in Human Subjects***

| Substance | Cumulative 48-h excretion (μmol) | | |
|---|---|---|---|
| | Smoked | Intravenous | Intranasal |
| AEME, Mean (*N*) | 0.85 (9) | 0.0077 (6) | 0.042 (6) |
| Range | 0.093–3.74 | 0–0.034 | 0–0.14† |
| Cocaine, Mean (*N*) | 1.58 (9) | 3.01 (7) | 4.14 (6) |
| Range | 0.25–4.25 | 0.74–7.34 | 1.32–13.9 |
| Benzoylecgonine, Mean (*N*) | 21.5 (9) | 51.4 (7) | 94.5 (6) |
| Range | 6.45–46.7 | 25.4–107 | 40.9–162 |
| Ratio, AEME/Cocaine, Mean (*N*) | 0.58 (9) | 0.01 (6) | 0.02 (6) |
| Range | 0.03–1.08 | 0–0.04 | 0–0.07 |

* Concentrations of cocaine and benzoylecgonien in urine were determined by the method of Jacob et al. (7).
† This subject was suspected of having used illicit cocaine during the period following nasal administration.

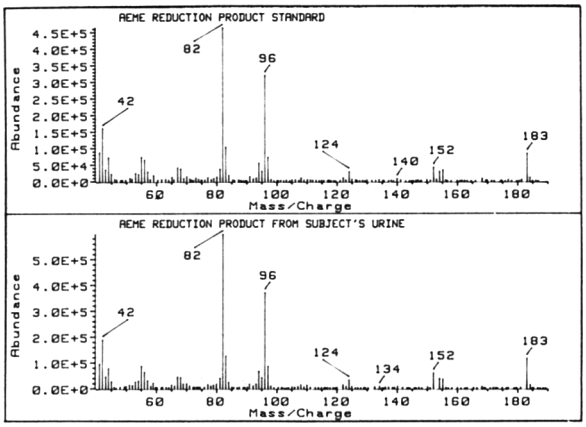


**Figure 4.** Mass spectra of the reduction product from AEME standard (upper panel) and from an extract of a cocaine smoker's urine (lower panel).

stered intravenously and intranasally, since a report suggested that AEME may be a cocaine metabolite (8). AEME was detected in only very small amounts or not at all in these sessions (Table II).

## Discussion

Our goal was to develop an analytical method suitable for determination of the low concentrations of AEME present in biological fluids several hours after cocaine smoking. For reasons of sensitivity and specificity, GC/MS was chosen as the method of analysis. However, thermal generation of AEME from cocaine in the heated injection port of a gas chromatograph would likely complicate the analysis, because cocaine would be expected in samples containing AEME. Indeed, an early peak due to AEME is often observed in gas chromatographic determination of cocaine. To overcome this problem, we developed a procedure for reducing the carbon-carbon double bond of AEME using sodium borohydride (as a hydrogen source) and a palladium catalyst which produces a saturated derivative 5 (Figure 2) easily separated from AEME by capillary GC, and in addition, is distinguished by its higher mass molecular ion used in detection by selected ion monitoring. With this procedure, less than 1% of the cocaine present is converted to the derivative 5. Both epimers of the reduction product derivative 5 were formed. The major epimer, which was used for quantitation and has the longer retention time (Figure 3), is presumably the 2-α-epimer resulting from addition of hydrogen to the less-hindered *exo*-face, although a definitive structural assignment cannot be made with the available data.

Urinary excretion of AEME in cocaine smokers has implications concerning problems associated with crack abuse. Because nothing is known about the pharmacology of AEME, the possibility that this compound plays a role in the pharmacology or toxicology of cocaine smoking must be considered. We are intrigued with the structural similarity of AEME to arecoline (Structure 3, Figure 2) and anatoxin (Structure 4, Figure 2), which suggests that AEME might have cholinergic activity (10-12). The amount of AEME absorbed by cocaine smokers is substantial, since the molar urinary concentrations approach

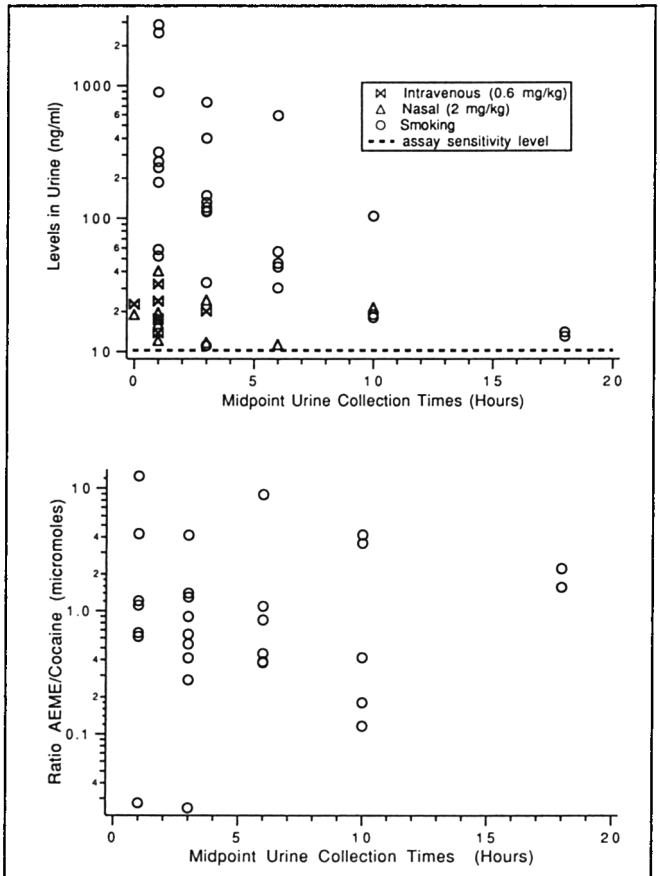


**Figure 5.** Concentrations of AEME in urine over 20 h folllowing cocaine smoking (upper panel); ratio of AEME/cocaine following cocaine smoking (lower panel).

those of cocaine. However, it is not possible to estimate exposure based only on urinary concentrations, because nothing is yet known about the metabolic disposition of AEME. Our smoking experiments were carried out in a research laboratory environment with relatively low doses of cocaine. Additional studies are necessary to determine the extent that AEME is absorbed by illicit users. The availability of a sensitive and specific analytical method should facilitate such studies.

It is also clear that little, if any, AEME is excreted in the urine of individuals administered cocaine by intranasal or intravenous routes. Whether the small amounts detected were an artifact or represent metabolic formation is unclear because small amounts ($\approx 0.3\%$) of cocaine present are converted to AEME under the conditions of the assay. The difference between smoking and other routes of cocaine administration is particularly dramatic when the molar ratios of AEME to cocaine excreted (0.58 for smoking, 0.01 for intravenous administration, and 0.01 for intranasal administration) are compared (Table III). This suggests that AEME could be a useful marker for recent cocaine smoking in forensic or toxicologic cases. Further research will be necessary to determine whether AEME plays a role in the pharmacology of crack use, and to validate its use as a marker for cocaine smoking.

A likely metabolite of AEME is anhydroecgonine (AE), formed by hydrolysis of the methylester group in a way analogous to the metabolism of cocaine to benzoylecgonine. Because benzoylecgonine is detectable in urine much longer and is present in much higher concentrations than cocaine, it would be reasonable to expect similarly higher and longer-lasting

urinary AE concentrations relative to AEME, making AE a better marker of cocaine smoking. This possibility is currently being explored in our laboratories.

## Acknowledgments

We are grateful to Christopher Brown, Maria Daehler, Patricia Buley, Andy Montalvo, Kaye Welch, Tina Melby, and Dolores Cannon for their assistance in this research. This work was supported in part of contract No. 271-87-8123 and Center grant No. DA01696 from the National Institute on Drug Abuse. The studies were carried out in part in the General Clinical Research Center at San Francisco General Hospital Medical Center (RR-00083) with support of the Division of Research Resources, National Institutes of Health.

## References

1. C.E. Cook, A.R. Jeffcoat, and M. Perez-Reyes. In *Pharmacokinetics and Pharmacodynamics of Psychoactive Drugs,* G. Barnett and C.N. Chiang, Eds., Biomedical Publications, Foster City, California, 1985, pp. 48–74.
2. M. Nova and C. Salemink. A model experiment in the study of cocaine base smoking. Isolation of methyl 4-(3-pyridyl)-butyrate. *Bull. Narc.* **36:** 79–82 (1984).
3. B.R. Martin, L.P. Lue, and J.P. Boni. Pyrolysis and volatilization of cocaine. *J. Anal. Toxicol.* **13:** 158–62 (1989).
4. C.L. Zirkle, T.A. Geissman, M. Bloom, P.N. Craig, F.R. Gerns, Z.K. Indik, and A.M. Pavlov. 3-Substituted tropane derivatives. I. The synthesis and stereochemistry of the tropane—3-carboxylic acids. A comparison of positional isomers in the tropane series. *J. Org. Chem.* **27:** 1269–79 (1962).
5. T. Lakarzewski and W.K. Jeffery. Impurities and artifacts of illicit cocaine. *J. Forens. Sci.* **25:** 499–507 (1980).
6. S.P. Findlay. The structure of anhydroecgonine ethyl ester. *J. Am. Chem. Soc.* **75:** 1033–35 (1953).
7. P. Jacob III, B.A. Elias-Baker, R.T. Jones, and N.L. Benowitz. Determination of benzoylecgonine and cocaine in biologic fluids by automated gas chromatography. *J. Chromatogr.* **417:** 277–86 (1987).
8. W.T. Lowry, J.N. Lomonte, D. Hatchett, and J.C. Garriot. Identification of two novel cocaine metabolites in bile by gas chromatography/mass spectrometry in a case of acute intravenous cocaine overdose. *J. Anal. Toxicol.* **3:** 91–95 (1979).
9. P. Jacob III, R.T. Jones, N.L. Benowitz, and A.T. Shulgin. Cocaine smokers inhale a pyrolysis product: Anhydroecgonine methyl ester. *Clin. Pharmacol. Ther.* **45:** 186 (1989).
10. U.A. Von Euler and B. Domeij. Nicotine-like action of arecoline. *Acta Pharm. Toxicol.* **1:** 263–69 (1945).
11. H.F. Campbell, O.E. Edwards, and R. Kolt. Synthesis of nor-anatoxin-a and anatoxin a. *Can. J. Chem.* **55:** 1372–79 (1977).
12. W.W. Carmichael, D.F. Biggs, and P.R. Gorham. Toxicology and pharmacological action of anabaena flos-aquae toxin. *Science* **187:** 542–44 (1975).

Manuscript received October 30, 1989;
revision received April 30, 1990.