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Application of a Pyroprobe to Simulate Smoking and Metabolic Degradation of Abused Drugs Through Analytical Pyrolysis*

ABSTRACT: Smoking of illicit drugs can produce unique metabolic biomarkers. Smoking conditions can be partially modeled via pyrolysis, a process that decomposes a chemical compound by extreme heat. Pyrolytic decomposition was found to be useful as a limited metabolic mimic in that analytical pyrolysis can be used to generate some of the same compounds produced by metabolic degradation. This project focused on the pyrolysis of cocaine and methamphetamine using a pyroprobe coupled with a GC/MS and more generally, potential applications of pyrolysis to forensic toxicology. Common diluents including lidocaine, caffeine, and benzocaine were pyrolyzed in mixtures with cocaine and methamphetamine. Correlations between pyrolytic and metabolic degradations revealed that this method has the capability to produce some of the reported metabolites such as norcocaine and cocaethylene for cocaine, and amphetamine for methamphetamine. The results demonstrate that analytical pyrolysis has the potential to identify some metabolic products and to supplement *in vivo* and enzymatic studies.

KEYWORDS: forensic science, toxicology, pyrolysis, drug analysis, metabolism

Pyrolysis is a rapid thermal decomposition process usually conducted under anaerobic conditions. Some previous methods of pyrolyzing abused drugs include using an apparatus to simulate smoking of a tobacco cigarette laced with the drug (1,2) or heating an aluminum boat (3). Disadvantages to these methods include analytical complexity and poor reproducibility due to imprecise temperature control. An analytical pyrolysis instrument, such as a pyroprobe, addresses the issue of thermal irreproducibility by heating samples in a controlled environment. Additional advantages are rapid sample analysis (*c*. 30 min) and minimal sample preparation. Pyroprobes have been used in forensic science for the pyrolysis of fibers, paints, photocopier toners, and polymeric materials (4–7), but to date, pyroprobes have not been widely used in forensic toxicology or solid dose drug analysis.

Pyrolysis can be particularly useful in smoked drug analyses, given the ability to mimic the smoking process and conditions. Smoked illicit drugs are a forensic concern because smoking may produce unique metabolic biomarkers. The advantage of pyrolysis in this role is that it is a simple, rapid, and inexpensive *in vitro* technique. Qualitative results are quickly obtained and can direct further research and analyses.

The present work used the utilization of a pyroprobe device coupled to a GC/MS for the pyrolysis of cocaine and methamphetamine. There were two primary goals to this project: first, to detect smoked biomarkers using this method and second, to compare the pyrolytic products to reported metabolic degradation products. The important pyrolytic product of cocaine is anhydroecgonine methylester (AEME) (8–11) while that of methamphetamine is 1-phenylpropene (12). The pyrolytic products obtained during the study were compared with products noted in the literature. Different ratios of drug and diluent such as lidocaine, benzocaine, and caffeine were analyzed to uncover any potential complications or interferences. The detected products were compared with the reported metabolites of cocaine and methamphetamine.

Materials and Methods

Materials

Cocaine and methamphetamine as hydrochloride salts, HPLC grade ethanol, benzocaine, lidocaine, and caffeine were all purchased from Sigma Chemical Co. (St. Louis, MO). The purity of cocaine and methamphetamine was confirmed by using FTIR. HPLC grade methanol was purchased from Fisher Science (Fair Lawn, NJ). Synthetic urine concentrate was purchased from RICCA Chemical Company (Arlington, TX).

Instrumentation and Conditions

Pyrolysis was performed utilizing a CDS Analytical 5150 pyroprobe (CDS Analytical Inc., Oxford, PA). The pyroprobe consists of four components: the pyroprobe, accessory, valve oven, and transfer line (Fig. 1). The sample was placed on a plug of quartz wool positioned inside a quartz tube. The quartz tube was placed inside the wire coil of the pyroprobe and inserted inside the accessory region of the pyrolysis unit. Helium was used to purge volatile components present at temperatures below the volatile temperature of the drugs. The pyrolysis temperatures were achieved in the valve oven via a controlled ramp rate (Fig. 2). Helium was used to carry the volatile pyrolytic products through the transfer line into the GC/MS. Pyrolysis and GC conditions for cocaine and methamphetamine are presented in Table 1. The MS consisted of an electron ionization/quadrupole with a 1 min solvent delay and a MS scan of 50–600 *m/z*.

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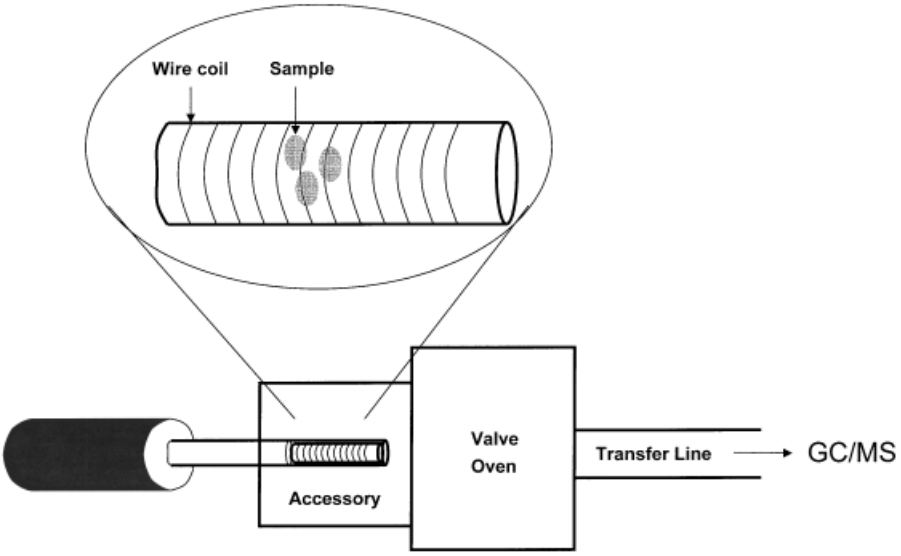

FIG. 1—*Schematic of a pyroprobe unit coupled to GC/MS.*

Drug Sample Preparation

Ten thousand parts per million stock solutions of cocaine and methamphetamine were made separately in methanol and ethanol. Methanol and ethanol were used for a comparative analysis as cocaine and methamphetamine are soluble in both organic solvents. In addition, using ethanol as a solvent allows for the formation of a cocaine transesterification product, cocaethylene (13). One microliter (10 µg) of the stock solution was injected inside the quartz tube and pyrolyzed. The solid sample analysis was performed by placing between 10 and 80 µg of the drug inside the quartz tube.

Cutting Agent/Drug Mixture Control

Ten thousand parts per million stock solutions of lidocaine, benzocaine, and caffeine were prepared separately. Lidocaine and benzocaine were dissolved in ethanol and caffeine in deionized water. A 1:1 mixture of the cutting agent with the drug was prepared to yield a total concentration of 5000 p.p.m. of each substituent. For lidocaine and benzocaine, 1 µL was injected into the quartz tube allowing for 5 µg of each substituent to be analyzed. Two microliter was used for the caffeine/drug analysis to yield 10 µg for analysis.

Results

The pyrolytic products of cocaine and methamphetamine have been previously reported and summarized in Table 2. Table 3 lists all the pyrolytic products obtained from the sample dissolved separately in methanol, ethanol, and in the solid form for comparison. The identification of the pyrolytic products produced (all simple molecules) was made using an NIST MS library search, supplemented with retention time comparison. The pyrolysis results revealed minor differences between using ethanol and methanol as solvents. Cocaethylene was a pyrolytic product of cocaine only when dissolved in methanol and amphetamine was produced only when methamphetamine was dissolved in ethanol. The reason for this difference has not been reported and is unclear. The pyrolytic products were reproducible ($n = 3$) in that the same mixture produced the same products with each run. However, area and peak height ratios were not reproducible and no quantitative analyses were attempted.

The common products found from literature reports and pyrolysis reported and observed from cocaine include AEME and benzoic acid. The additional experimental pyrolytic products include norcocaine, cocaine, and cocaethylene. The pyrolysis of methamphetamine using this method produced two of the reported

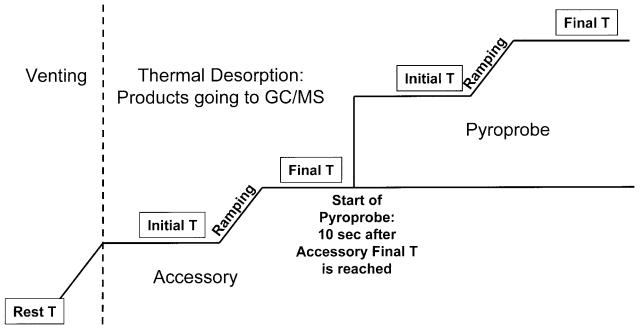

FIG. 2—*Representation of the pyroprobe temperature program.*

TABLE 1—*Pyrolysis and GC conditions.*

Pyrolysis Conditions		
	Cocaine	Methamphetamine
Accessory temperatures		
Rest	50°C	50°C
Initial	90°C 1 min	50°C 1 min
Ramp rate	100°C/min	100°C/min
Final	350°C 15 min	100°C 2 min
Pyroprobe temperatures		
Initial	50°C 1 sec	100°C 1 sec
Ramp rate	20°C/sec	20°C/sec
Final	750°C 10 sec	800°C 10 sec
GC conditions: Elite-5 capillary column (30 m × 0.25 mm ID with a film thickness of 0.25 µm), Clarus 500 GC/MS (Perkin Elmer, Wellesley, MA)		
Initial temperature	70°C	50°C (1 min)
Ramp rate 1	15°C/min	20°C/min
Temperature 2	130°C	150°C (5 min)
Ramp rate 2	8°C/min	30°C/min
Temperature 3	210°C	250°C (2 min)
Ramp rate 3	10°C/min	
Temperature 4	290°C	

TABLE 2—*Reported pyrolytic products of cocaine and methamphetamine.*

Cocaine	References	Methamphetamine	References
Anhydroecgonine (AE)	(8)	Amphetamine*	(1)
Anhydroecgonine methylester (AEME)	(8)	Dimethylamphetamine	(14)
Benzoic acid	(9)	Phenylacetone	(15)
Methyl-4-(3-pyridyl)-butyrate	(11)	1-phenylpropene	(12)
Methyl benzoate	(11)	*N*-acetyl-methamphetamine*	(1)
Methyl cycloheptatrienecarboxylate isomers	(11)	*N*-formyl-methamphetamine*	(1)
N-methylbenzamide	(11)	*N*-propionyl-methamphetamine*	(1)
Norecgonidine	(16)	*N*-cyanomethyl-methamphetamine*	(1)
Norecgonidine methylester	(16)	Bibenzyl*	(17)

*Note that for methamphetamine, the pyrolytic products were produced in the presence of tobacco.

TABLE 3—*Pyrolysis results of cocaine and methamphetamine utilizing a pyroprobe/GC/MS.*

Drug	Solid Form	In Methanol	In Ethanol
Cocaine	Benzoic acid	Benzoic acid	Benzoic acid
	AEME	AEME	AEME
	Norcocaine	Norcocaine	Norcocaine
	Cocaine	Cocaine	Cocaine
	Cocaethylene	Cocaethylene	
Methamphetamine	Bibenzyl	Bibenzyl	Bibenzyl
	Benzene	Benzene	Benzene
	Toluene	Toluene	Toluene
	Ethylbenzene	Ethylbenzene	Ethylbenzene
	Styrene	Styrene	Styrene
	1-phenylpropene	1-phenylpropene	1-phenylpropene
	Methamphetamine	Methamphetamine	Methamphetamine
			Amphetamine

AEME, anhydroecgonine methylester.

pyrolytic products, 1-phenylpropene, bibenzyl, and amphetamine. Benzene, toluene, ethylbenzene, styrene, and methamphetamine were also produced.

A 1:1, 1:2, and 2:1 (wt:wt) ratio of cocaine:methamphetamine were analyzed for any additional products produced by the possible interaction between the drugs. For the 1:1 mixture, 5 μg of each drug was pyrolyzed while in the other cases, 10 and 5 μg for the 2:1 and 1:2 were used respectively. No additional pyrolytic products were produced other than what was detected from single drug analysis. Similarly, the products of a 1:1 ratio of drug: cutting agent (lidocaine, benzocaine, and caffeine) are shown in Table 4. Pyrolysis of benzocaine and caffeine only produced an analyte peak; however, pyrolytic products were detected with lidocaine as shown.

Discussion

The ability of a pyroprobe to produce pyrolytic products in mixtures was demonstrated and the products observed using pyrolysis mirrored those found in biological fluids. There were no unique pyrolytic products found from interactions between cocaine and methamphetamine. Results depicted in Table 4 show no detectable pyrolytic products for cocaine when mixed with lidocaine or benzocaine. AEME was produced when cocaine was mixed with caffeine. The methamphetamine: cutting agent mixtures did produce most of the pyrolytic products of methamphetamine, even at the lower concentrations.

A simplified comparison between analytical pyrolysis and metabolism is illustrated in Fig. 3. Both processes are degradations in which a larger molecule is broken down into smaller stable molecules. In the case of metabolism, the process can be described as oxidative decomposition catalyzed enzymatically, the effect of which is to decrease the energy barrier, E_a, of the reaction of interest as shown in the figure. Aerobic biodegradation by enzymes is selective and specific and generally results in increased water-soluble products. In contrast, anaerobic pyrolysis is not selective because high temperatures are applied to a chemical compound in which the decomposition is nonspecific. Analytical pyrolysis provides sufficient thermal energy to overcome the activation energy barriers of multiple degradation pathways (Fig. 4). Therefore, it is reasonable to expect some common products. More importantly for the present work, analytical pyrolysis provides energies comparable with those observed when a drug is ingested by smoking.

Figure 5 shows a comparison of metabolic products of cocaine to the experimentally determined pyrolytic products using the pyroprobe/GC/MS method. Norcocaine and cocaethylene were detected from cocaine, but the other reported metabolites, benzoylecgonine,

TABLE 4—*Pyrolytic products of the diluents, and diluents with cocaine and methamphetamine.*

Sample	Pyrolytic Products
Lidocaine	Phendimetrazine, lidocaine
Lidocaine+cocaine	Phendimetrazine, lidocaine, cocaine
Lidocaine+methamphetamine	Ethylbenzene, styrene, bibenzyl, methamphetamine, lidocaine
Benzocaine	Benzocaine
Benzocaine+cocaine	Benzocaine, cocaine
Benzocaine+methamphetamine	Ethylbenzene, styrene, bibenzyl, methamphetamine, benzocaine
Caffeine	Caffeine
Caffeine+cocaine	AEME, caffeine, cocaine
Caffeine+methamphetamine	Ethylbenzene, styrene, bibenzyl, methamphetamine, caffeine

AEME, anhydroecgonine methylester.

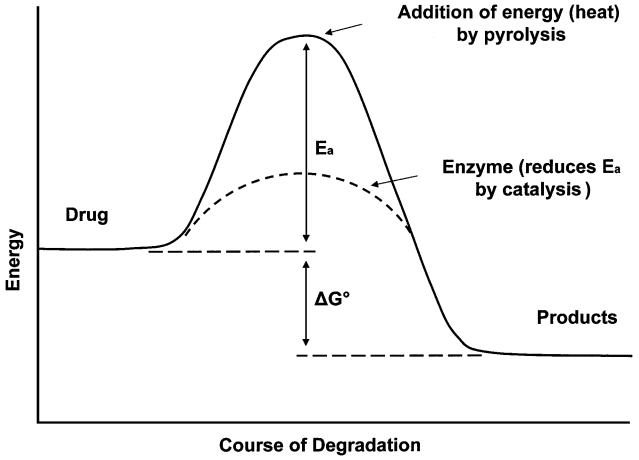

FIG. 3—*Plot of the energy profile from drug (reactant) to products.*

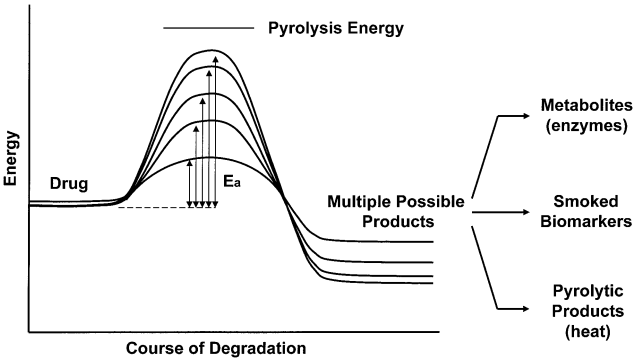

FIG. 4—*Plot of the multiple energy pathways leading to various products.*

and ecgonine methylester, were not present. Figure 6 summarizes the possible metabolic and pyrolytic products from the decomposition of methamphetamine. Pyrolysis of methamphetamine produced amphetamine, while 4-hydroxynorephedrine, 4-hydroxyamphetamine, 4-hydroxymethamphetamine, hippuric acid, and norephedrine were not detected after several attempts. The percent areas were determined for the cocaine and methamphetamine pyrolytic products and are shown in Figs. 5 and 6. The percentages were calculated by dividing the peak area of each pyrolytic product into the largest peak area in the chromatogram.

These results are not surprising. The cytochrome P450 enzymes breakdown compounds differently than a thermal decomposition. All enzymes do not perform the same type of reaction; therefore, these differences in enzyme function allow for different degradation pathways. In addition, the metabolites that were not detected

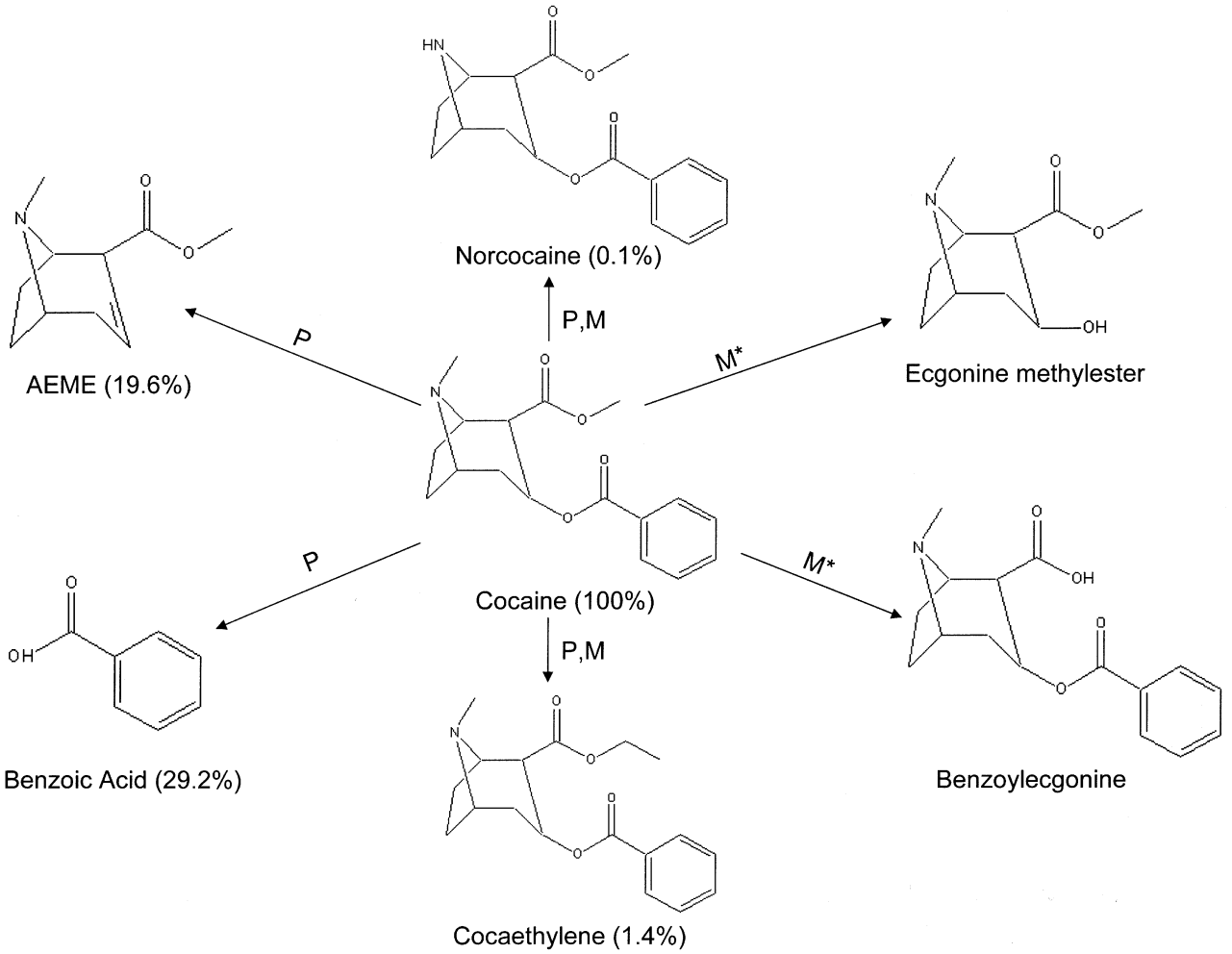

FIG. 5—*Cocaine, its pyrolytic products and percent areas obtained by a Pyroprobe/GC/MS (P) and metabolites (M) (*not detected by this method). Metabolites were reported in Lewis et al. (8).*

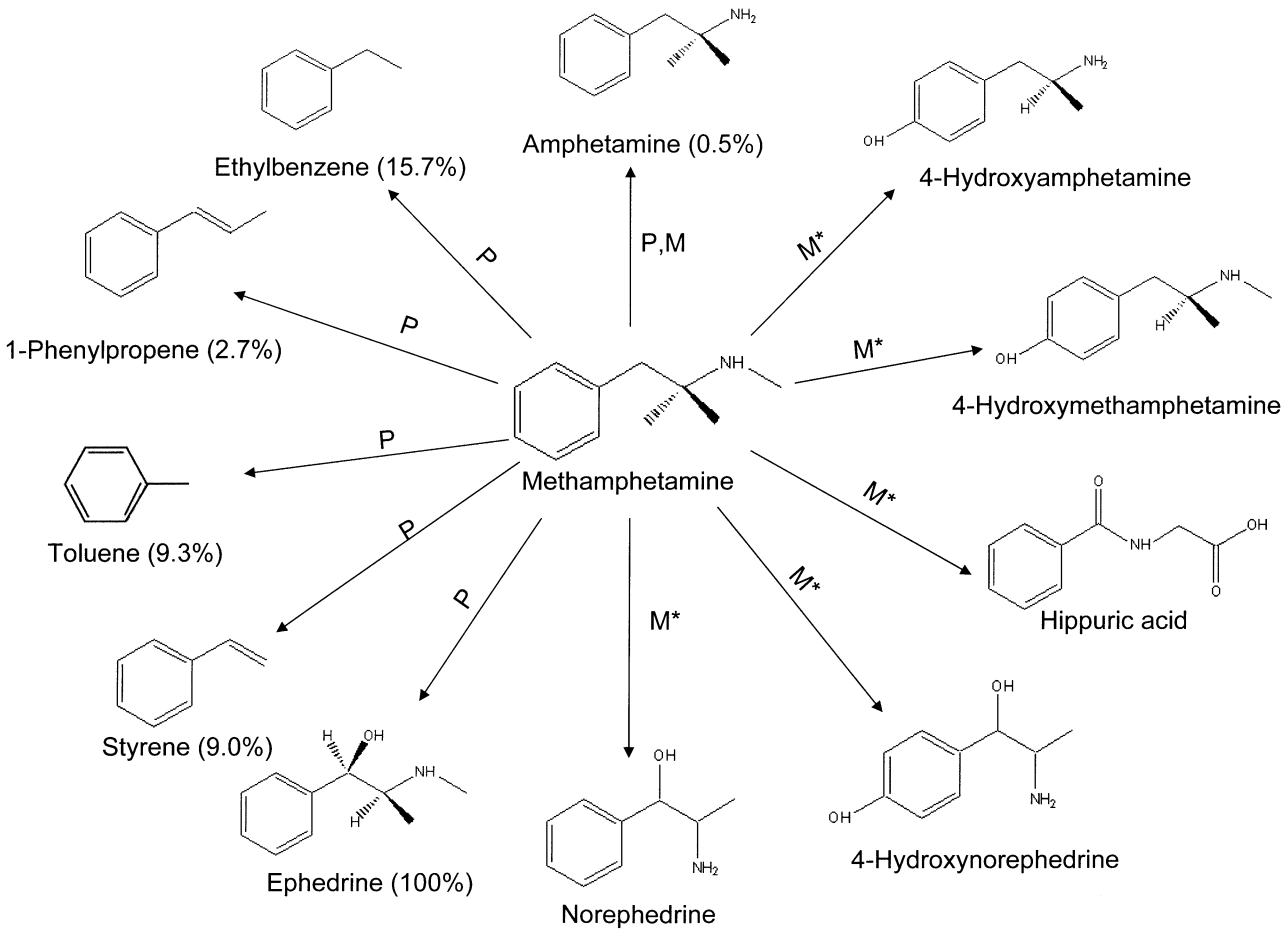

FIG. 6—*Methamphetamine, its pyrolytic products and percent areas obtained by a Pyroprobe/GC/MS (P) and metabolites (M) (*not detected by this method). Metabolites were reported in Caldwell et al. (18).*

by pyrolysis also share a similarity in that oxygen and water are necessary for these products to form. Providing conditions similar to metabolism (i.e., oxidative conditions) may increase the chances of producing the metabolites. Therefore, introducing the reactant gas, air, will allow for water and oxygen to be present during pyrolysis. This is currently under investigation.

To be useful to forensic toxicology, it must be amenable to biological matrices. Experiments comparing the pyrolytic products produced from cocaine and methamphetamine dissolved in synthetic urine versus organic solvents were conducted. A 10,000 p.p.m. stock solution of cocaine and methamphetamine were prepared in synthetic urine. A 1 μL sample of the stock solution was pyrolyzed under the same GC and MS conditions stated previously. The data revealed that the pyrolytic products obtained in synthetic urine were more abundant than in methanol or ethanol. The reason for this is not clear. However, the method has potential for a screening method and rapid process for predicting possible metabolic products in forensic toxicology.

Future work utilizing the pyroprobe/GC/MS involves extending the idea of modeling metabolism by introducing air into the pyroprobe. The presence of oxygen and water during thermal degradation may provide better conditions for producing the unseen metabolites. The ability to simulate smoking and metabolism by pyrolysis will be beneficial to forensic toxicologists and for the future application of this method to other abused drugs.

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