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α-Benzyl-*N*-Methylphenethylamine (BNMPA), an Impurity of Illicit Methamphetamine Synthesis: III. Detection of BNMPA and Metabolites in Urine of Methamphetamine Users

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Abstract

Eighty urine specimens collected from drug rehabilitation programs, which had been screened by immunoassay and confirmed positive by gas chromatography–mass spectrometry (GC–MS) for methamphetamine, were further analyzed for α-benzyl-*N*-methylphenethylamine (BNMPA) and its urinary metabolites, *N*-demethyl-BNMPA, diphenyl-2-propanone (DP2P), diphenyl-2-propanol, *p*-OH-*N*-demethyl-BNMPA, and *p*-OH-BNMPA. BNMPA is an impurity of illicit methamphetamine synthesis. Analysis of BNMPA and its metabolites was performed by quantitative GC–MS following β-glucuronidase hydrolysis, liquid–liquid extraction, and derivatization with heptafluorobutyric anhydride. Two urine specimens contained detectable amounts of BNMPA and/or its metabolites. One contained trace amounts (greater than the limit of detection but less than the limit of quantitation) of *N*-demethyl-BNMPA and DP2P, as well as 0.04 mg/L *p*-OH-*N*-demethyl-BNMPA. The other contained trace amounts of BNMPA, *p*-OH-BNMPA, and *p*-OH-*N*-demethyl-BNMPA, as well as 0.03 mg/L *N*-demethyl-BNMPA. Prior to analyzing these urine specimens, pure reference material of *p*-OH-BNMPA was made available, and analysis confirmed our previous tentative identification of *p*-OH-BNMPA as a major metabolite of BNMPA. Detection of BNMPA or its metabolites in biological samples may serve as a marker of illicit methamphetamine administration.

Introduction

Abuse of amphetamines reached epidemic proportions during the late 1940s through 1950s when these compounds were readily available by prescription (1). The Controlled Substances Act of 1970 stringently regulated the manufacture of these stimulants and forced manufacturers to decrease sales to retail pharmacies (2). Because methamphetamine is easily synthesized in crude laboratories, a dramatic increase has occurred in the illicit production and abuse of methamphetamine over the past several years. Endemic areas for this increase include the Pacific coast states, Hawaii, and other Pacific rim countries such as Japan and Korea (3).

There are several popular methods of illicit methamphetamine synthesis (4,5). It is well known that side reactions and incomplete conversions ("impurities of manufacture") can easily occur in most of the illicit synthetic methods and that "street chemists" rarely, if ever, produce a pharmaceutically pure product. Impurities of manufacture are numerous and are characteristic of a particular synthetic method. They have been extensively reviewed elsewhere (6–9). Few impurities have been studied in vivo, and limited information exists concerning their pharmacology and toxicology (10,11).

Because impurities can be characteristic of a particular synthetic method, their presence in seized samples or their detection in biological samples from methamphetamine users can be used to monitor the sales of precursor chemicals, to group seized compounds to common sources of illicit production, and to provide links between manufacturers, dealers, and users.

α-Benzyl-*N*-methylphenethylamine (BNMPA) is an impurity arising from the synthesis of methamphetamine via the Leukart reaction using phenyl-2-propanone synthesized from phenylacetic acid (12). We predicted the major metabolites of BNMPA to be *N*-demethyl-BNMPA, diphenyl-2-propanone (DP2P), diphenyl-2-propanol (DP2P-OH), *p*-OH-BNMPA, and *p*-OH-*N*-demethyl-BNMPA. We have previously synthesized BNMPA, *N*-demethyl-BNMPA, and DP2P-OH and, in a controlled excretion study in humans, confirmed that they, as well as what we believed to be the *p*-OH compounds were, in fact, the major metabolites of BNMPA (13,14).

This communication presents the results of the analysis for BNMPA and its metabolites in 80 urine specimens collected from clients at substance abuse treatment programs. These samples had been screened by immunoassay and confirmed positive by gas chromatography–mass spectrometry (GC–MS) for methamphetamine or amphetamine. Prior to analyzing these urine specimens, pure reference material of *p*-OH-

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BNMPA was made available, and analysis confirmed our previous tentative identification of *p*-OH-BNMPA as a major metabolite of BNMPA (14).

Materials and Methods

Reagents and chemicals

DP2P was purchased from Eastman Chemical Co. (Rochester, NY). All other chemicals were purchased from Aldrich Chemical Co. Solvents were high-performance liquid chromatographic grade and purchased from Fisher Chemical/Fisher Scientific (Fair Lawn, NJ). Heptafluorobutyric anhydride (HFBA) was purchased from Regis Chemical Co. (Morton Grove, IL). D-Amphetamine-d_3 sulfate and β-glucuronidase (type H-2, *Helix pomatia)* were purchased from Sigma Chemical Co. (St. Louis, MO). Amphetamine-d_3 was used as the internal standard for all GC–MS procedures.

BNMPA and *N*-demethyl-BNMPA were synthesized as previously described (13). *p*-OH-BNMPA was a gift from Dr. Richard Glennon, Department of Medicinal Chemistry, Medical College of Virginia. The structure, as the oxalate salt (melting point, 180–182°C), was confirmed in his laboratory with proton nuclear magnetic resonance. We further confirmed the structure of the underivatized and HFBA-derivatized compound in our laboratory with GC–MS. A chromatogram of the derivatized synthetic compound, as well as the synthetic standards of BNMPA and its other metabolites, is pictured in Figure 1.

GC–MS analysis

Prior to extraction of BNMPA and its metabolites, urine specimens were hydrolyzed with β-glucuronidase as previously described (14). The analytes were then extracted with *n*-butyl chloride and derivatized with HFBA, also previously described (14). All GC–MS analyses for BNMPA and its metabolites were performed on a Hewlett-Packard 5890 GC equipped with an HP-1 capillary column (12 m × 0.2-mm i.d; 0.33-µm film thickness) connected to a Hewlett-Packard 5971A mass selective detector (11). For BNMPA and its metabolites, the lower limit of detection (LOD) and the lower limit of quantitation (LOQ) were 0.003 and 0.025 mg/L, respectively. Calibration curves were linear from 0.025 to 0.5 mg/L. Methamphetamine and amphetamine were analyzed by the GC–MS HFBA derivatization method of Thurman et al. (15).

Urine samples

Frozen urine specimens were obtained from PharmChem Laboratories, Inc. (Menlo Park, CA). Only specimens that yielded positive amphetamine results by Emit® II Amphetamine/Methamphetamine assay (Syva Co., San Jose, CA) in our laboratory were analyzed for BNMPA metabolites. Forty of the urine specimens were received in December 1993 and represented specimens that had been collected throughout 1993. The second group of 150 urine specimens were received in September 1994 and represented specimens collected during the 2 months preceding their shipment.

Results

Of the first group of urine specimens received in December 1993, all were found to contain methamphetamine and amphetamine. All of these samples were analyzed for BNMPA and its metabolites. Two urine specimens from this group of 40 contained detectable amounts of BNMPA and/or its metabolites. One urine specimen contained trace amounts (greater than the LOD but less than the LOQ) of *N*-demethyl-BNMPA and DP2P, as well as 0.04 mg/L *p*-OH-*N*-demethyl-BNMPA. This sample contained 45 mg/L methamphetamine and 1.2 mg/L amphetamine. The other urine specimen contained trace amounts of BNMPA, *p*-OH-BNMPA, and *p*-OH-*N*-demethyl-BNMPA, as well as 0.03 mg/L *N*-demethyl BNMPA. The methamphetamine and amphetamine concentrations were 25 and 3.1 mg/L, respectively.

Of the second group of 150 urine specimens received in September 1994, only 88 screened positive for amphetamines by Emit II in our laboratory. These 88 urine specimens were then analyzed for BNMPA and its metabolites. Of these, only 40 yielded positive methamphetamine or amphetamine results or both by GC–MS (cutoff, 0.5 mg/L). None of these urine specimens, collected at least 1 year after the original group, contained BNMPA or its

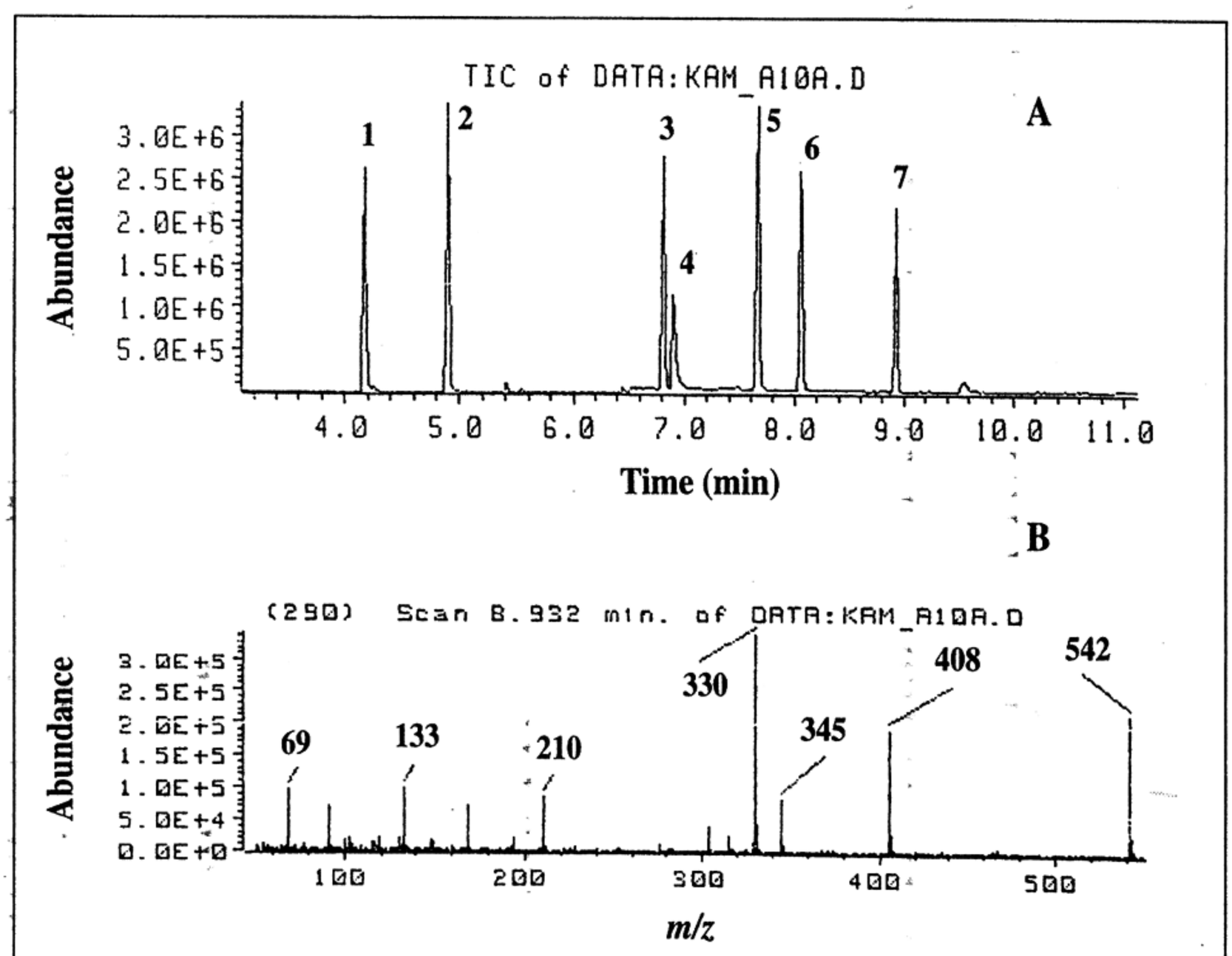

Figure 1. (A) Total-ion chromatogram of BNMPA and its metabolites. Peaks: 1, amphetamine-d_3; 2, methamphetamine-d_5; 3, diphenyl-2-propanol; 4, diphenyl-2-propanone; 5, *N*-demethyl-BNMPA; 6, BNMPA; and 7, *p*-OH-BNMPA. (B) Electron-impact mass spectra of *p*-OH-BNMPA.

metabolites, although the methamphetamine concentration was as high as 77 mg/L. The methamphetamine negative urine specimens were found to contain massive amounts of other phenylisopropanolamine derivative drugs.

Previously, we had tentatively identified *p*-OH-BNMPA as a major urinary metabolite of BNMPA, following the oral administration of 5 mg to a human subject (14). Our tentative identification was based upon expected metabolism of BNMPA, the resultant mass spectrum of the HFBA derivative of the unknown metabolite, and the apparent increase in concentration of the unknown compound following β-glucuronidase or acid hydrolysis prior to extraction. The retention time and mass spectrum of the HFBA derivative of reference material *p*-OH-BNMPA (Figure 1) matched the previously collected data from our dose study (14) and that of the two *p*-OH-BNMPA positive urine specimens in this study.

Discussion

Methamphetamine laboratories comprised more than 50% of all laboratories seized by the Drug Enforcement Administration (DEA) during a 45-month period ending in September 1981 (4). The most popular methods of illicit synthesis at that time required phenyl-2-propanone (P2P) as an initial reactant. In 1981, P2P became a Schedule II controlled substance. Clandestine laboratories were then forced to synthesize P2P from phenylacetic acid. The most common synthetic by-product present in P2P prepared from phenylacetic acid is DP2P (5). If DP2P contaminated P2P is used to synthesize methamphetamine via the Leukart reaction, BNMPA is a major contaminant (12).

Recently, difficulties in obtaining P2P or other precursors necessary for the reductive amination methods of synthesis, combined with the increased availability of (–)-ephedrine and (+)-pseudoephedrine both in this country and the Far East, has resulted in a conversion of clandestine synthesis of methamphetamine to ephedrine reduction methods (16). Methamphetamine is synthesized by reductive cleavage of the hydroxyl group in ephedrine, either by thionyl chloride or hydriodic acid and red phosphorus (5). In 1993, the DEA participated in the seizure of 270 clandestine laboratories in the United States, of which 218 were methamphetamine laboratories. The ephedrine reduction method was used in 81% of these methamphetamine laboratories, whereas the P2P methods were used in only 16% (17).

Only two of the 80 urine specimens containing methamphetamine in this study collected during 1993 and the summer of 1994 contained BNMPA and/or its metabolites. This is most likely due to the recent change in illicit synthesis from reductive amination to ephedrine reduction methods. In March 1994, the DEA proposed to make all regulated transactions of ephedrine, regardless of size, subject to the reporting and recordkeeping requirements of the Chemical Diversion and Trafficking Act of 1988 (CDTA) (18). This would subject all transactions involving bulk ephedrine and single entity ephedrine drug products to the applicable provisions of the CDTA. CDTA has already made the acquisition of commercially produced hydriodic acid much more difficult. The illicit price of a gallon of hydriodic acid has since increased sharply. Lack of availability and increased price has caused violators to seek alternative sources of hydriodic acid and to apply different processes to the clandestine manufacture of methamphetamine (19). The tightening of controls on ephedrine availability may shift clandestine laboratories back to reductive amination methods of methamphetamine synthesis. Should this occur, impurities of synthesis such as BNMPA may become significant in identifying the illicit source of methamphetamine. We have demonstrated that detection of BNMPA and its metabolites in urine may serve as a marker for administration of illicit methamphetamine synthesized via the Leukart reaction.

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