Identification of di(β-phenylisopropyl)amine as the main ingredient in illicit amphetamine tablets

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Summary. - The identification of di(β-phenylisopropyl)amine found as the main ingredient in several sets of amphetamine tablets sold on the illicit market in Rome, Italy, during 1999-2000 is described. The identification was achieved by examining the ultraviolet and nuclear magnetic resonance spectral properties as well as the chromatographic, gas chromatographic, and mass spectrometric data. The molecular structure of the ingredient showed a close analogy to the amphetamine and could very likely produce similar pharmacological activity. A preliminary test on the metabolic pathway of di(β-phenylisopropyl)amine performed on rats, suggests its biotransformation to amphetamine.

Key words: di(β-phenylisopropyl)amine, amphetamines, GC-MS, NMR, designer-drugs.

Introduction

Since the early ‘90s amphetamines and their ring- and N-substituted derivatives have frequently been observed in the illicit market of recreational drugs.

On a weekly basis our laboratory performs analysis for the Court of Law of Rome on the illicit material seized by the Italian State Police. As a result, it is possible to have a picture of the local illicit drug market. The authors have observed that psycho stimulant drugs such as cocaine and amphetamines have increased in popularity among young people [1].

Over the past year, it has also been noticed that illicit tablets, even though engraved with the usual symbols previously encountered in the so-called “ecstasy”, did not contain methylenedioxyamphetamine but rather amphetamine, often associated with various amounts of caffeine.

Furthermore, the laboratory recently received a few sets of tablets with different symbols, containing a small amount (ranging from 5 to 15%) of amphetamine, some caffeine and another molecule that accounted for the same or even for a greater amount than the amphetamine. Other colleagues working in various Italian forensic laboratories received similar sets.

Each set accounted for hundreds of tablet for a total of a few thousand throughout Italy.

Based upon the above, the Court of Law of Rome authorized us to use some of the seized tablets for scientific research.

The objective of the research was to identify the unknown molecule in order to see what class of drugs it belonged and if it was already listed in the controlled substances schedule of the Italian law.

As a first step we started with the identification of di(β-phenylisopropyl)amine according to the procedure developed by this laboratory for the characterization of unknown compounds which included: extraction procedure, UV spectrophotometry, thin layer chromatography (TLC), gas chromatography (GC) coupled with flame ionization detector (GC-FID), mass spectrometry (GC-MS) both in electron impact mode (EI) and chemical ionization (CI), and magnetic nuclear resonance spectroscopy (1H NMR) [2].

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Parole chiave: di(β-fenilisopropil)ammina, amfetamine, GC-MS, NMR, designer-drugs.

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Once the chemical structure was identified and the analogy with the benzphetamine was highlighted, a preliminary test was also performed in order to verify its metabolic conversion to produce amphetamine.

The results reported here are confined to a single set of tablets representative of those that were sold in the illicit market. They were pink in color, engraved with a bird on one side and scored on the other side (Fig. 1).

**Experimental**

All solvents and reagents were of analytical reagent grade (J.T. Baker - Deventer, Holland). Standards of amphetamine hydrochloride, caffeine hydrochloride and sugars (sucrose, lactose, glucose and fructose) were obtained from the collection of standards of the Laboratory of Forensic Toxicology of the University Tor Vergata, Rome.

Deuterated chloroform used as a solvent for FT-1H NMR was purchased from Sigma-Aldrich, Milan, Italy. UV spectra of the methanolic solutions were obtained with a Varian DMS 200 UV-Vis. spectrophotometer at a wavelength range between 190 and 360 nm.

TLC was conducted on Silica Gel 60 F254, 0.25 mm pre-coated plates (Merck). The mobile phases for the separation of the basic compounds were: cyclohexane:toluol:diethylamine (75:15:10) [3] and ethanol:strong ammonia solution (100:1.5). The detection was achieved under an UV light at 254 nm. The solvent system for the carbohydrates was: n-butanol:ethyl acetate:glacial acetic acid:2-propanol:water (14:38:14:23:11) and the location reagent was a mixture of sulfuric acid 2.5% in ethanol and resorcinol 5% in ethanol.

GC-FID analyses were performed on a Varian 3300 gas chromatograph fitted with a wide-bore column SE-30, 15 m x 0.53 mm i.d., 1.2 μm film thickness (Alltech); carrier gas: N2; flow rate: 5 ml/min; oven temperature programmed between 80 °C (1 min isothermal) and 250 °C at a rate of 15 °C/min and 1 min final hold time; injection port: 250 °C, detector: 280 °C.

GC-MS (EI) analyses were performed on a Hewlett Packard 5890 gas chromatograph coupled with a Hewlett Packard 5973A EI Mass Selective Detector, fitted with a HP-5 capillary column 30 m x 0.25 mm i.d., 0.25 μm film thickness; carrier gas: He; flow rate: 0.8 ml/min; oven temperature programmed between 80 °C (2 min isothermal) and 280 °C with 20 °C/min rate and 1 min final hold time; injection port: 250 °C; split ratio 1:20; source temperature: 280 °C; EI mode: 70 eV electron energy.

For GC-MS (CI) a Varian GC3800-Saturn 2000 ion trap mass spectrometer, fitted with a Chrompack CP-sil 8 CB capillary column, 30 m x 0.25 mm i.d., 0.25 μm film thickness was used; carrier gas, Helium; oven temperature programmed between 150 °C (1 min isothermal) and 290 °C with 10 °C/min increment rate and 6 min final hold time; injection port: 260 °C; split ratio 1:20. MS parameters: reagent gas CH4; Sec/Scan 0.6; background Mass 45; reagent ion ejection 9 Volts; CI reaction storage level 13 m/z; ARC target 5000.

1H NMR spectra were performed on a Varian Mod. Gemini 200 MHz in the pulse Fourier Transform mode. Samples were dissolved in deuterated chloroform and the chemical shifts were reported in unit ppm from tetramethylsilane (TMS) as internal reference.

**Sample preparation**

The average weight of one tablet was about 245 mg and the average dimension was: diameter 8 mm, thickness 4 mm.

Aliquots of powdered tablets were dissolved in methanol, centrifuged and submitted to UV spectrophotometry and to thin-layer chromatography (TLC) for amphetamines and for sugars.

For GC analysis, amounts of 20 mg of each powder were placed into screw-capped tubes and dissolved in 2 ml of distilled water. Then 1M NaOH solution to pH 12-14 was added (the pink color of the solution turned yellowish). Alkaline aqueous solutions were extracted with 2 x 5 ml of ethyl acetate. The upper organic layers were then transferred into other screw-capped tubes for the GC (FID and MS using both EI and CI (positive ionization) analysis.

The separation by TLC of the tablet ingredients was performed in order to isolate the unknown compound for submission to 1H NMR and UV spectrophotometry.

The investigated compound was scraped off the plate and extracted from the silica gel using cyclohexane. Prior to solvent evaporation colloidal silica was removed by centrifugation and filtration through a membrane filter.

![Fig. 1. - Photograph of the seized tablets with the bird logo.](image-url)
**Biotransformation test**

The purification of the di(β-phenylisopropyl)amine from the amphetamine and the caffeine, also present in the tablets, was achieved by multiple TLC using a non-toxic mobile phase (ethanol/ammonia 100/1.5) to obtain a product suitable for the test.

The compound was scraped off the plate, dissolved in ethanol, and separated from the adsorbent by centrifugation and filtration. The absence of amphetamine residual traces was tested by GC-MS (SIM mode).

A single dose of 10 mg of the purified compound (equal to 28.5 mg/kg) was given orally to three rats that were transferred in metabolic cages. On the basis of previous pharmacological studies on rats and mice the amount of di(β-phenylisopropyl)amine used in this single dose test was well below the DL$_{50}$ calculated in mice (106 mg/kg) [4].

The administered dose was estimated to correspond, on the basis of a theoretical 100% absorption and metabolic conversion, to a maximum of 5.3 mg of amphetamine (DL$_{50}$ in rats = 180 mg/kg s.c.).

Twenty-four hours urine samples were collected prior to and after dosing. The samples were then extracted at pH 14 with tert-butyl-methyl ether and analyzed by GC-MS in EI mode (SCAN and SIM).

**Results**

**Identification of the compound**

The UV spectra obtained from the methanolic solutions of the powdered tablets showed the spectrum of the caffeine. The UV spectrum of the compound isolated by TLC and dissolved with methanol showed an amphetamine-like profile with two maximums of absorbance at 254 and 259 nm wavelength.

TLC separation for basic compounds with the non-polar mobile phase showed Rf values of 0.05 for caffeine, 0.10 for the amphetamine, and 0.68 for the investigated compound.

The TLC purification with the ethanol/ammonia solution gave Rf values of 0.50 for the caffeine, 0.62 for the amphetamine, and 0.83 for the investigated molecule.

The TLC for carbohydrates indicated the presence of lactose and sucrose as diluents.

GC-FID analysis of the alkaline extracts from the tablets, performed with a wide-bore column, showed three significant peaks. Two of them were identified as the amphetamine and the caffeine. The third peak (Rt 9.61) corresponded to the unknown molecule (Fig. 2).

The formation of a Shiff base by reaction of the compound with acetone was attempted. No derivatives were obtained. The negative result indicated the lack of a primary amine group in the molecule.

Extracts of the tablets analyzed in EI mode with capillary column confirmed the presence of amphetamine and caffeine and showed two unresolved peaks at about Rt 11 min. The peaks had closely related fragmentation patterns not included in the Mass Spectra database. This was interpreted as the probable existence of isomeric forms in the molecule.

In order to obtain information about the molecular weight of the compound a positive CI analysis using methane as a reagent gas was performed.

The spectra showed that the molecular weight was 253 AMU due to the presence of an ion at m/z 254 corresponding to the m/z $[M + H]^+$ and of the typical adduct $m/z$ 294 $[M + C_3H_5]^+$ (Fig. 3).
The ion m/z 238 in the EI spectrum could thus be referred to as the m/z [M - 15] (Fig. 4). The study of the fragmentation pattern in addition to the information provided by the other analytical techniques employed, led us to form the hypothesis that the compound was di(β-phenylisopropyl)amine.

The cleavage in β to the nitrogen caused the base peak ion m/z 162 and the ion m/z 91 (benzyl group); the carbon-nitrogen cleavage from the base peak produced the ion m/z 119 [5]. The ion m/z 44 (CH3 - CH = N+H2) resulted from an α,β cleavage with hydrogen rearrangement [6].

In order to confirm the predicted structure the 1H NMR spectrum of the compound was compared with the spectrum of amphetamine free-base standard under the same analytical condition (Fig. 5).

The 1H NMR spectrum of the compound was consistent with the presence of two benzene rings (10 protons), two methyl groups (6 protons), two methylene (4 protons), and two methine groups (2 protons).

The two methyl groups were represented by two doublets, in agreement with the predicted isomeric forms likely to be obtained in clandestine laboratory synthesis. The presence of two carbon chiral centers in the molecule was, indeed, consistent with the formation of two enantiomeric forms and one meso form.

Biotransformation test

The chromatograms obtained from the extracts of the three urine samples collected after dosing, showed the peak of the amphetamine as well as the peak of the unchanged di(β-phenylisopropyl)amine.

In each chromatogram, the area of the amphetamine peak exceeded the area of the unmodified compound.

No other peaks related to the original molecule were identified.

Conclusions

The analysis of the ingredients of the tablets sold in the illicit market in Italy allowed for the identification and characterization of the di(β-phenylisopropyl)amine found along with some amphetamine and caffeine.

Fig. 4. - GC-MS total ion current (TIC) chromatogram of the alkaline extract of the tablets. EI mass spectra associated with the two unresolved peaks. Analytical conditions are described in the text.

Fig. 5. - 1H NMR spectra of: a) standard amphetamine free-base; b) di-β-(phenylisopropyl)amine. Analytical conditions are described in the text.
The molecule was already known to be an impurity occurring as intermediate during illicit amphetamines synthesis [8, 9].

In the present case, the di(β-phenylisopropyl)amine amount per tablet was quite large (equal to or even higher than the amphetamine) so we decided to verify whether it could be considered as a prodrug.

Due to the structural similarity between the di(β-phenylisopropyl)amine and the benzphetamine (N-methyl-N-bezyl-β-phenylisopropylamine), it could be predicted that one amphetamine molecule could originate by its in vivo metabolism.

Although only a single dose test on a few animals was performed [10, 11], the preliminary results appear to confirm that an in vivo transformation to produce amphetamine occurred. The urinary products were, indeed, amphetamine and a small amount of the unchanged drug (about 10% respect to amphetamine).

In conclusion, the clandestine tablets seized in the Italian market contained an active, non-controlled molecule that seems to be an amphetamine prodrug. As a result, the pharmacological activity of these tablets cannot be restricted only to their low amphetamine content.

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