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Short communication

GC/MS based identification of skunk spray maliciously deployed as "biological weapon" to harm civilians☆,☆☆

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ABSTRACT

Our laboratory has been asked to elucidate the origin of a strong "toxic smell" present in a prominent politician's office, private house and motorcar. This stinky and pungent atmosphere has caused serious nausea and vomiting to several individuals. Urine samples were collected from the persons presenting symptoms of nausea for toxicological analysis. Drops, paper and cotton swabs of an oily liquid found at the implicated places were submitted by police to our laboratory for investigation. Methanol extracts of the drops were acetylated for gas chromatography/mass spectrometry (GC/MS) analysis in the electron impact mode; the cotton and paper swabs were analysed using headspace-gas chromatography/mass spectrometry (HS-GC/MS). The GC/MS analysis of the acetylated methanol extracts revealed that the major peaks of the chromatogram could be attributed to 2-methylquinoline, to 2-quinolinemethanethiol, to S-2-quinolinemethyl thioacetate, to 2-phenylethanethiol, to bis(E)-2-butenyl disulphide and to bis(3-methylbutyl) disulphide. Several volatile sulphur-containing compounds have been identified with the HS-GC/MS system. Detailed examination of the spectra as well as GC/MS analysis of commercially available skunk secret allowed us to relate the identified compounds to those present in the defence spray of skunks. No health sequels were observed for any of the persons implicated in this case.

1. Introduction

Our laboratory has been contacted by phone with complains from several individuals presenting nausea and vomiting due to a stinky and pungent smell when entering a prominent politicians' office and her private house. Soon afterwards the politician and her staff showed up in our institute for toxicological advice, alleging that they were the victims of a "chemical attack" by unknown offenders. Nausea and vomiting taken a part, no other symptoms were observed. Urine samples for toxicological analysis were immediately collected from persons presenting these symptoms.

Meanwhile a lawsuit had been filed and small drops of yellow oily liquids have been discovered by police in the letter box of the politician's office, at the front door of her private house, on the engine bonnet and in the air filter of her motorcar. These items found at the implicated places were submitted by police to our laboratory.

All collected specimens have been analysed by using different GC/MS and HS-GC/MS methods for the identification of toxicants and drugs. "General unknown screening" methodology of oily or aqueous liquids is done using GC/MS and HS-GC/MS analysis. The GC/MS is performed after liquid/liquid extraction and acetylation of the dry extract. The HS-GC/MS analysis is done on a small aliquot of the sample without pre-treatment. The GC/MS allows detecting most volatile organic compounds such as drugs, drugs of abuse, pesticides, poisons and pollutants. The HS-GC/MS analysis allows the detection and quantification of low molecular weight substances as alcohols, solvents or gasoline constituents. For identification the experimental spectra were compared to a commercially available library. When available, the identified compounds were also compared to pure standard solutions.

2. Materials and methods

2.1. Chemicals

Analytical grade methanol, 2-propanol, ethyl acetate and hexane were obtained from Lab-Scan Analytical Sciences (Lab-Scan Ltd., Dublin, Ireland). Acetic acid anhydride, hydrochloric acid and pyridine were purchased from Merck (Darmstadt, Germany). Ammonium hydroxide was purchased from VEL (Louvain, Belgium).

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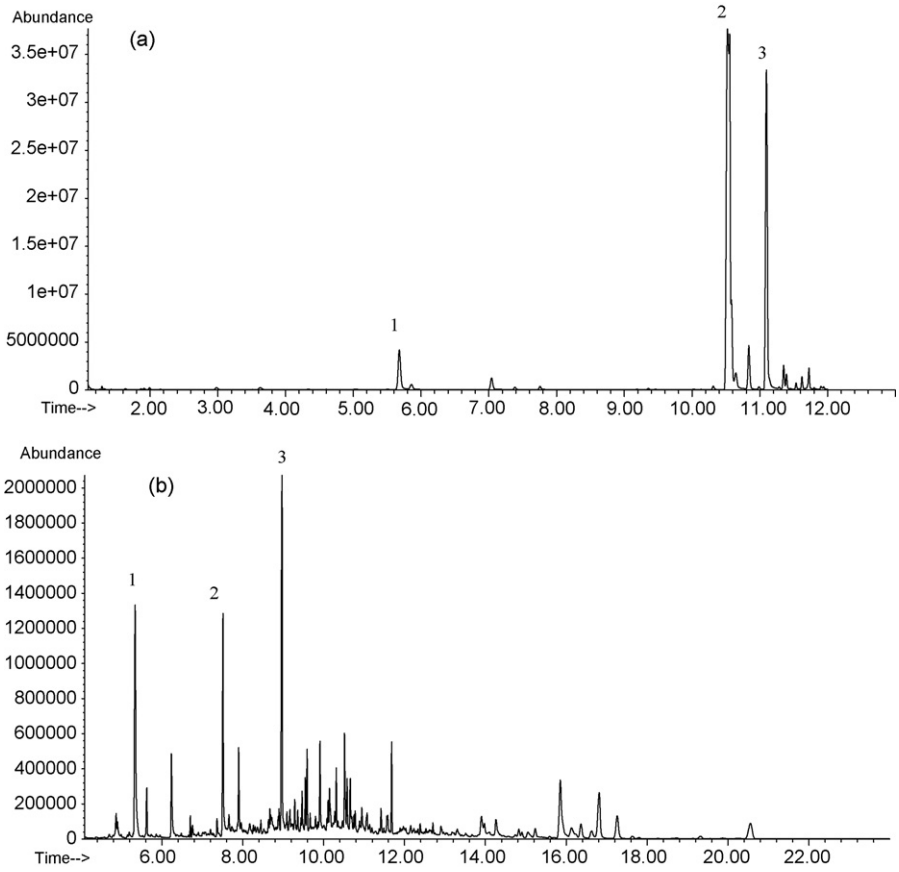

Fig. 1. (a) Chromatrogramme of HS/GC–MS analysis (1: rt = 5.70 min, (E)-2-butene-1-thiol, 2: rt = 10.5 min, S-(E)-2-butenyl thioacetate, 3: rt = 11.1 min, S-3-methyl-butanyl thioacetate, rt = retention time). (b) Chromatogramme of methanol extract of a swap collected from the motor car (1: rt = 5.3, 2-methylquinoline, 2: rt = 7.5 min, 2-quinolinemethanethiol, 3: rt = 9.0 min S-2-quinolinemethyl thioacetate. Other important identified compounds were located at rt 10.0–11.5 min, rt = retention time).

2.2. Specimen preparation

2.2.1. Urine samples

General unknown screening using GC/MS was performed as described earlier [1]. Briefly, 500 μL of concentrated hydrochloric acid were added to 2 mL of urine and heated at 90 °C for 20 min, then hydrochloric acid was neutralized using NaOH. The solution was separated in two aliquots, each aliquot was buffered (pH 5.5 and pH 9.5, respectively) and extracted using 3 mL of 2-propanol/hexane (15/85, v/v). Evaporation of the extracts was done at 40 °C under a gentle flow of nitrogen and acetylation of the residues was done using 100 μL of acetic acid anhydride in the presence of 100 μL of pyridine. The reaction mixture was heated at 90 °C for 20 min and then evaporated to dryness as described above. The final residues were taken up in 100 μL of ethyl acetate/methanol (90/10, v/v). One microlitre was injected into the GC/MS system in splitless mode for analysis.

HS-GC/MS analysis using 500 μL was performed for the detection of volatile organic compounds.

2.3. Drops and swabs

The oil drops were collected as such or using cotton or paper swabs. They were placed for 30 min in methanol for desorption of the unknown compounds. Methanol was evaporated and the residue was acetylated for GC/MS analysis for the detection of non-volatile compounds as described below. Cotton and paper swabs were placed into a 10 mL HS vial (Agilent Technologies, Diegem, Belgium), and screened for the presence of volatile compounds using HS-GC/MS.

2.4. GC/MS analysis

Substance separation was achieved using a 6890N gas chromatograph from Agilent Technologies (Belgium) fitted with a 12 m Ultra-2 capillary column from HP (Agilent Technologies, Belgium) with 0.2 mm internal diameter and 0.33 μm film thickness. Helium was used as carrier gas at a flow rate of 0.5 mL/min. The initial column temperature was 70 °C (2 min); ramp 30 °C/min to 220 °C, ramp 5 °C/min to 240 °C and finally 30 °C/min to 280 °C, this temperature was maintained for 6 min. The injector temperature was 260 °C. Electron impact (EI) mass spectra were recorded with a mass selective detector (5971A-Series II) from Agilent Technologies (Belgium). The temperature of the MSD transfer line was 280 °C and the ionization voltage was 70 eV. The temperature of the ion source was 230 °C. All spectra were recorded in scan mode from m/z 50 to 650.

2.5. HS-GC/MS analysis

The headspace sampler was a G1888 model (Agilent Technologies) connected with a 6890N network GC system. The GC system was equipped with a DB-624 column (30 m × 0.32 mm × 1.8 μm) from JW Scientific at 90 °C during the whole run. The injector temperature was 200 °C, column temperature was held constant at 90 °C and mobile phase (helium) flow rate was 1.0 mL/min. Injection was performed in split mode and the split ratio was 15.8/1. Spectra were recorded from m/z 25 to 300.

All experimental GC/MS and HS-GC/MS spectra were identified using commercial libraries for toxicological analysis ("Mass Spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants and Their Metabolites", Wiley–VCH, and "NIST 05 Mass Spectral Library",

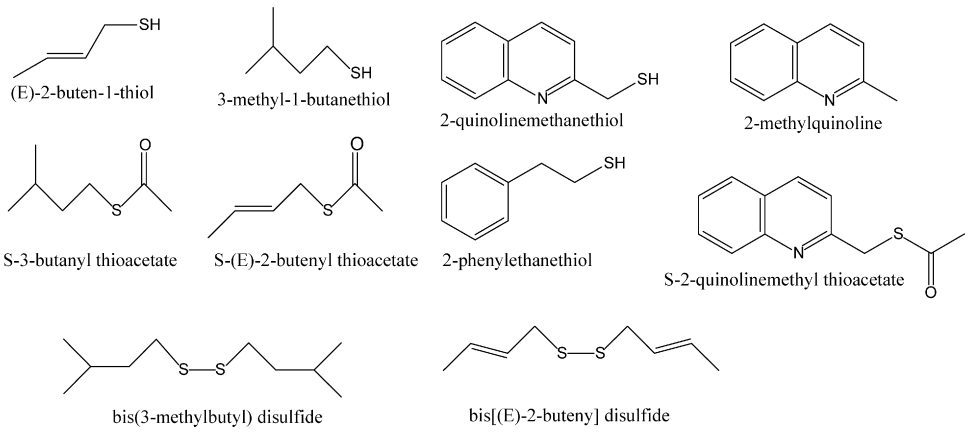

Fig. 2. Major compounds identified in drops and swabs.

NIST). Also, all spectra were manually analysed for the identification of characteristic functional groups and structures.

3. Results

3.1. Urine samples

In the urine samples it was not possible to detect any volatile toxic substances which could explain the symptoms experienced by the patients. Usual comprehensive drug screening was also negative.

3.2. Drops and swabs

In HS-GC/MS analysis (Fig. 1a) the following volatile compounds have been identified: (E)-2-butene-1-thiol, S-(E)-2-butenyl thioacetate, 3-methyl-1-butanethiol, S-3-methyl-butanyl thioacetate. Carbon disulphide (CS_2) has been detected in other samples (results not shown).

In the GC/MS chromatogram (Fig. 1b) of the methanolic extracts major peaks could be attributed to: 2-methylquinoline, 2-quinolinemethanethiol, S-2-quinolinemethyl thioacetate, 2-phenylethanethiol, bis(E)-2-butenyl disulphide and bis(3-methylbutyl) disulphide. The 4-methylquinoline isomer (known as an industrial chemical) has been excluded by comparison with the other methylquinoline metabolites which are all for the 2-isomer family. Some minor peaks present in the chromatogram could not be identified. No other common toxic substances have been detected. MS spectra of all compounds have been described in scientific literature and are represented in Fig. 2.

Due to the lack of reference compounds no quantification of the identified compounds could be performed. Relative peak intensities of the identified compounds were slightly different in the individual specimens as they were sampled at different places, at different sampling times and as these constituents are all presenting individual volatility.

4. Discussion

In a first approach some of the identified spectra were believed to be related to industrial chemicals. However after discussion with industrial manufacturers we could exclude this hypothesis. Indeed the presence of different thiols and thioacetates brought us to the idea that these substances may be natural products; e.g. they were identical to those believed to cause the vile odour of anal gland secretion of skunks [2–5].

Proportions of the sulphur-containing constituents of the excretions of these skunks are species dependent and also not totally identical. One major class of compounds produced by skunks is composed of thiols as: (E)-2-butene-1-thiol, 3-methyl-1-butanethiol, phenylethanthiol and 2-quinolinemethanethiol in different amounts.

A second major class is constituted by thioacetates as: (S-(E)-2-butenyl thioacetate, S-3-methyl-butanyl thioacetate, S-2-quinoline thioacetate). Thioacetates are easily hydrolyzed by water to stinky thiols. Thiols are primarily responsible for the odour, while thioacetates are haemolysing agents.

A third type of compound is an alkaloid named 2-methylquinoline, metabolically related to 2-quinolinemethanethiol and S-2-quinolinemethyl thioacetate. A fourth class of compounds are sulphides: carbon disulphide, bis(E)-2-butenyl disulphide and bis(3-methylbutyl) disulphide. All these compounds have been detected in the drops and swabs collected in the politician's house, office and motor car.

Some skunk scent gland extracts are commercially available in the US and Australia and may also be purchased in Europe using the internet. In one of these specimens that we could obtain ("SkunkShot") and declared as "synthetic skunk oil 8% in petrolatum" we identified dibutyl disulphide, n-butylisopentyl disulphide, 3-methyl-1-butanethiol, 1-butanethiol, 3-butanethiol and several aliphatic alkanes. Some of these compounds have been detected in our case. As not all of our compounds are present in the available commercial skunk shot we suppose that another commercial defensive spray was used in the present attack. The drops may also have been prepared from a natural source.

It is well known that deodourizing skunk spray can be easily produced by oxidizing the thiols into sulphonic acids using any oxidizing agent (e.g. hydrogen peroxide with sodium hydrogencarbonate or liquid laundry bleach (eau de Javel)). There are anecdotic reports claiming that skunk defensive spray can be toxic, if a high dose is inhaled, ingested or sprayed directly on skin of pets [6]. Immediate symptoms may include vomiting, diarrhoea, convulsions and shock.

These attacks are never fatal but persistent anaemia [7], partial loss of renal function and mouth ulcers may develop. This constellation of symptoms is known as "Skunk Toxic Shock Syndrome". Headache, nausea, vomiting likely owing to the strong odour, mild irritation of mucosa have been described and have also been observed in our case. For pulmonary oedema and CNS depression very high levels are required. For butanethiol a threshold limit value ("TLV") at 2 mg/m^3 in occupational health had been established in the USA. In accidental spoilage cases concentrations of 200–2000 mg/m^3 have been observed. It is well known that olfac-

tive perceptibility levels for thiols are about fractions of $\mu g/m^3$ so that an acute intoxication risk is extremely low. For this reason we concluded that there were no serious health effects to be expected in our case.

Even if we succeeded in identifying many compounds, one must be aware that the techniques used also have limitations and ingredients of the drops and swaps submitted to our laboratory may also have been missed: the maximum m/z which has been scanned was 650 amu so higher molecular weight compounds will not be detected, thermally unstable compounds (i.e. proteins) may break down in the injector or on the GC column and finally some compounds may not extracted by liquid/liquid extraction (i.e. organophosphorus compounds or quaternary ammoniums salts).

5. Conclusions

The presence of most common toxic substances has been excluded after performing a general unknown screening with GC/MS and HS-GC/MS used in combination with commercial libraries for systematic toxicological analysis. A literature review and manual MS spectra interpretation allowed us to identify compounds identical to those produced by skunks for their defence. Thus liquid/liquid extraction combined to GC/MS and HS-GC/MS showed to be powerful analytical tools for identification of unusual compounds.

The present study shows that compounds produced by skunks may be used in "chemical/biological threats" to generate psychological stress because of their repugnant smell. However, these compounds are otherwise relatively harmless when present at low concentrations. In our case the repugnant smell lasted for several days. During this period nobody could enter the office or home of the politician. No health sequels were observed for any of the persons implicated in this incident. As far as we know the offender(s) of this incident has (have) never been identified.

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