CASE REPORT

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Fatal Poisoning from Nicotiana Glauca Leaves: Identification of Anabasine by Gas-Chromatography/Mass Spectrometry

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ABSTRACT: Death of a worker occurred after ingestion of unknown amounts of Nicotiana glauca G leaves. The leaves were cooked after having been mistakenly considered to be spices of a type which grow in Thailand. After ingestion, two Thai workers collapsed, one with asystolia. Resuscitation efforts were successful only for one of the victims. A GC/MS method was used for the identification of anabasine as the main constituent in the leaves, food extract, blood, and the urine of the deceased. Lacking a standard, it was necessary to interpret the GC/MS spectrum to identify anabasine and establish its presence.

KEYWORDS: forensic science, nicotiana glauca, tobacco tree, poisoning, fatal poisoning, gas chromatography, mass spectrometry (GC/MS)

Nicotiana glauca G (tree tobacco), a slender evergreen sticky shrub, is an annual of nightshade (Solanaceae) growing in many parts of the world. In Israel the plant is found along walls, on debris or growing along sandy regions. The plant is from Nicotiana species, the entire plant, including its large leaves and yellow long tubular flowers, contains 4–5% of structurally-related alkaloids, which are narcotic and poisonous.

Anabasine (neonicotine), a highly toxic piperidine-related alkaloid, is the major alkaloid of Nicotiana glauca G (1). It is structurally similar to nicotine (Fig. 1), and it is present in numerous Nicotiana species (e.g., Nicotiana tabaccum) as well as in other families (2,3). Anabasine is also found as a minor tobacco alkaloid together with nicotine and nornicotine. Its content varies among various parts of the plant: the woody material concentration of anabasine is low, whereas the leaves and bark content is quite high. Anabasine is commercially produced in Russia as an insecticide from Anabasis aphylla (4,5).

Teratogenic effects by consumption of Nicotiana glauca were documented in the offspring of cattle (6), sheep (7), and swine (8), and were attributed to the ingestion of anabasine. These deformities are clinically similar to those caused by maternal consumption of coniine (poison hemlock) such as carpal flexure, cleft palates, arthrogryposis of the forelimbs (9,10) and curvature of the spine. Anabasine, together with other cigarette-smoke compounds were suggested to affect endocrine function through aromatase inhibition (11). The inhibition of acetylcholinesterase (AChE) by anabasine and other related compounds was reported (12), and the pyrrolidine ring was considered to be important in binding to the acetylcholinesterase (AChE).

Incubation of human granulosa cell with the combination of nicotine, cotinine and anabasine, resulted in inhibition of progesterone synthesis, suggesting a cytotoxic effect of these alkaloids (13).

Very few cases of human ingestion of boiled Nicotiana glauca leaves have been reported in either fatal (14) or near-fatal (15) incidents. In most of the cases the poisoning occurred when the patients mistook a wild tobacco plant for an edible green.

The content of anabasine in different plants of Nicotiana species varies. It has been determined by gas chromatography and by infrared spectroscopy (1) to be about 0.1% of the plant content, although some ranges of 0.08–0.82% have been reported (16). In Israel Police Analytical Chemistry Laboratory anabasine was determined by GC to be about 0.2% of the content of 12 collected Nicotiana glauca samples. Anabasine was analyzed in smokers' urine (15) and from autopsy organs (14) by a gas chromatography/mass spectrometry (GC/MS).

Although cases of anabasine poisoning are common in both humans and animals, fatal poisonings have been seldom described in human. The authors present a fatal case of anabasine poisoning in which anabasine was identified by using a gas chromatography/mass spectrometry (GC/MS) method for the detection of anabasine from Nicotiana glauca G, and from the poisonous food, after extraction. Large quantities of anabasine were identified in the residue from a basic extract of the urine and in smaller quantities in the blood by GC/MS, and were seen on thin-layer chromatography (TLC). The mass spectrum of anabasine is briefly discussed.

Materials and Methods

Extraction Procedure for GC/MS and TLC

A. Leaves and Food—Nicotiana glauca leaves and stalks were found in the possession of the deceased. To create a simulated meal, leaves and stalks (50 g) were cooked under the instruction of

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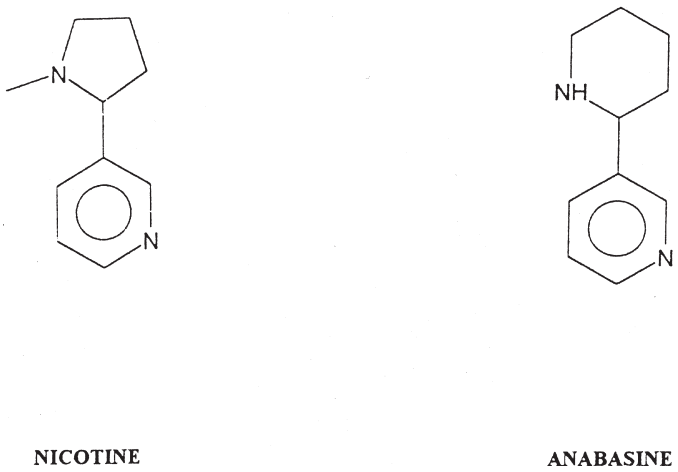

NICOTINE ANABASINE

FIG. 1—*Chemical structures of nicotine and anabasine.*

the Thai survivor (case A), with the original ingredients as had been done previously.

Ground Nicotiana glauca leaves (25 g) and the simulated cooked meal (100 g) were separately placed in 100 mL glass vials. After adding 30 mL of chloroform, the vials were capped and sonicated for 10 min. A portion of both extracts was filtered through a 0.45 mm disposable filters for TLC screenings and for the GC/MS analyses.

B. Urine and Blood—Approximately 5 mL of methylene chloride-isopropanol (50:50) were added to a 3 mL aliquot of body fluids in a conical tube and 2.5 mL of phosphate buffer pH 10 (40% K_2HPO_4) in distilled water. The mixture was vortex-mixed for 5 min, centrifuged at 2500 g for 5 min, and cooled in dry-ice-acetone bath to freeze the aqueous layer. The organic phase was removed into a new tube and evaporated to dryness in a vacuum evaporator at 60°C. 50 μL methanol were added to the residue. 2 μL of the solution were analyzed by GC/MS.

Thin Layer Chromatography (TLC)

Extracts from Nicotiana glauca leaves and stalks, urine, and blood together with standard solution of nicotine were spotted on a silica gel 60 plate (E. Merck and Co.) and were developed in two eluent systems: toluene:acetone:ethanol:ammonium hydroxide (45:45:7:3), and dioxane:xylene:ethanol:ammonium hydroxide (40:40:5:5). After drying, the spots were visualized under UV light (254 and 360 nm) and sprayed with a saturated solution of ninhydrin to give a blue color for the mentioned extracts (R_f 0.3). Under these conditions, nicotine (R_f 0.5) gives a brown color. Nicotine anatabine, nornicotine, and metanicotine were not detected.

Gas Chromatography/Mass Spectrometry

The data for GC/MS were generated using a Hewlett-Packard (HP) 5890 gas chromatograph connected to an HP 5970B Mass Selective Detector (MSD), operated in the scan mode and controlled by an IBM computer using 3.11 version Window software. The instrument performs 70 electron volt EI ionization Mass Spectrum. A DB-5 bonded-phase fused silica GC column, 15 m by 0.25 mm internal diameter with a 0.25 μm film thickness of poly (5% diphenyl-95%-dimethylsiloxane) was used. Split injection (1 μL) was done at 220°C, at split ratio of 1:9. The column temperature was programmed from 50° to 290°C at a rate of 25°C/min. Helium gas was used as a carrier with flow rate of 1 mL/min.

Case Report

Nicotiana glauca leaves were mistakenly considered as spices that grow in Thailand. Unknown amounts were added to the food and cooked. Two Thai males ate at the same time from this cooked meal.

Case A—A 52-year-old Thai male was admitted to the intensive care unit due to a self-poisoning after eating a very small amount (three spoons) from his food. A few minutes later he threw up and went to sleep. Two hours later he felt severe headache, nausea and vomiting. External examination by paramedics showed no abnormal findings. After preliminary treatment he recovered gradually and was discharged by the paramedics.

Case B—A 46-year-old Thai male ate leftovers of the same food. Two hours later and one hour before admission to the hospital, he felt nausea, headache, hypersalivation, vomiting, tachycardia, tachypnea, hypertension, and hyperthermia symptoms which are related to possible exposure to tobacco products. Very shortly thereafter the patient collapsed with cardiac arrest. External examination by paramedics yielded no abnormal findings. Toxicological screening for alcohols and acidic, basic, and neutral drugs was negative. Treatment was directed toward removing the poison and counteracting or controlling the patient's signs. The patient was treated with intravenous fluids and respiratory support. Despite resuscitation efforts, circulation could not be restored and he died in intensive care six days after admission. An autopsy was not performed. Neither nicotine nor its metabolites were found in the urine or the blood extracts; however; anabasine was identified in large quantities in the residue from a basic extract of the urine and in smaller quantities in the blood by GC/MS, and it was also seen on a thin-layer chromatography (TLC).

Results and Discussion

Most of the difficulties encountered in the analysis of tobacco alkaloids arise from their acid-base properties. To facilitate the extraction of the alkaloids from a tobacco sample, the solution of the extraction has to be acidic to convert the basic alkaloid compounds into water-soluble protonated forms. Nicotine, nornicotine, and anabasine are very soluble in chloroform, which is used in some procedures to extract alkaloids from tobacco (17). Although better results are obtained with methanol, the authors preferred to work with chloroform to prevent the extraction of sugars (18).

The chloroformic extracts of the leaves, the cooked meal, the residue from the basic extracts of the urine and the blood were screened by TLC for anabasine. The TLC patterns from the case material were similar to the extract from the plant. Lacking an anabasine standard, there was no way to confirm which of the spots was anabasine. However, spots with different R_f and different colors from standard nicotine were observed.

Analyses of the extracts from the leaves, the meal, and from the basic residue of the urine and blood were performed by gas chromatography/mass spectrometry (GC/MS) to attempt to identify the unknown alkaloids. Quantitation analysis was not performed. The analysis was first intended to help the diagnosis to use the proper medical treatment and later for the determination of the cause of death. Both chromatograms gave a peak at Rt 6.09 min. The mass

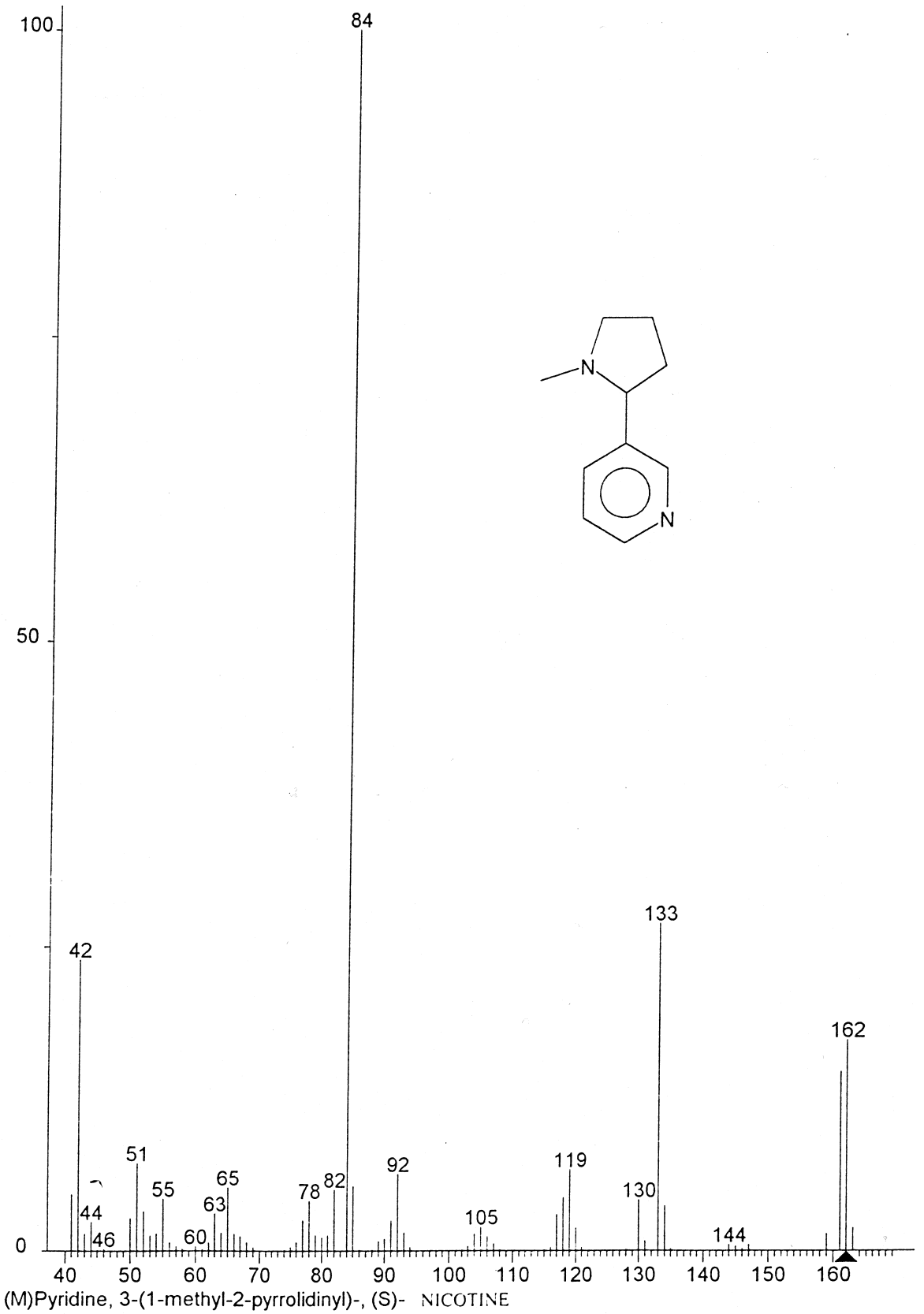

FIG. 2—*Mass spectrum of nicotine.*

spectrum was similar but not identical to that of nicotine (Fig. 2). Since a standard of anabasine was not available it was necessary to interpret the GC/MS spectrum to identify anabasine and establish its presence. It was identified by the authors as nicotine's isomer, anabasine (Fig. 4). The EI mass spectra of both nicotine and anabasine were previously reported (19). An apparent molecular ion of m/z 162 was present for both compounds, but additional fragments with different abundance were present in the anabasine spectrum. The M-1 ion is formed largely by loss of the hydrogen atom at C-2 affording the conjugated immonium species. A base peak of both nicotine (Fig. 2) and anabasine (Fig. 3) occurs at m/z 84 [M-78], corresponding to the loss of a pyridine ring through ∝-cleavage of the molecular ion at the C-2 position. The ions at m/z 133 [M-29], m/z 119 [M-43], and m/z 105 [M-57] must involve a hydrogen migration. Elimination of C_4H_8 gives the ion at m/z 106 [M-56], whereas the ion at m/z 105 [M-57] is generated probably by expulsion of a butyl radical. These two ions are significant to anabasine. The peaks at m/z 92, m/z 65, and m/z 51 in the mass spectrum of nicotine and anabasine are typical to the cleavage of the pyridine ring (20).

Poisoning of anabasine is characterized by severe nicotine-like toxicity, primarily neuromuscular blockage and respiratory failure. Nicotine alkaloids are rapidly absorbed from the oral and gastrointestinal mucous as well as the respiratory mucous and the skin. Apparently, the gastric absorption of nicotine alkaloids from tobacco taken by mouth is delayed, so that vomiting (nicotine alkaloids induce vomiting by a direct stimulation of the emetic chemoreceptor trigger zone) caused by the central effect of the initially absorbed fraction removes much of the tobacco remaining in the stomach before a fatal dose is absorbed. This action could explain the spontaneous emesis of the patient described in case A.

In both cases the patients exhibited a delay of about two hours in the onset of symptoms probably due to a slower gastric absorption of anabasine from the tobacco leaves. In case B the deceased did display many of the usual toxic symptoms including salivation, diaphoresis, headache, dizziness, mental confusion, marked weakness, faintness, hypertension, and paralysis of the respiratory muscles, and he finally collapsed with cardiac arrest. In reported fatal cases of nicotine poisoning, death usually occurred within one hour of the onset of symptoms. Supportive care should begin as early as possible to improve the prognosis of the patient. In case B, the intoxication of the deceased apparently occurred after ingestion a single toxic dose in a short period. Although he was provided with respiratory support and intravenous fluids, he was not able to recover. On the other hand, the patient in case A ate smaller amounts of the cooked plant, and clinical intoxication occurred in a mild manner.

Tissue distribution of anabasine had been investigated in human (14) and in animals (21). Except for the high levels of anabasine in the gastric contents, these results show a tendency for the drug to accumulate in kidneys, where it undergoes primary elimination, and then in the brain, the heart, and the lungs. Lower levels were measured in the blood. Although an autopsy was not performed in case B, anabasine was seen in TLC and identified by GC/MS in large quantities in the urine and relatively in small quantities in the blood of the victim. These findings were confirmed by the literature (14), although the compounds were not quantified. In animal studies, anabasine was found to be less potent than nicotine in blocking spinal reflexes and other nicotinic effects (22). However, its lethality is three times greater than that of nicotine in rabbits and guinea pigs. Death always was due to respiratory failure (14,23).

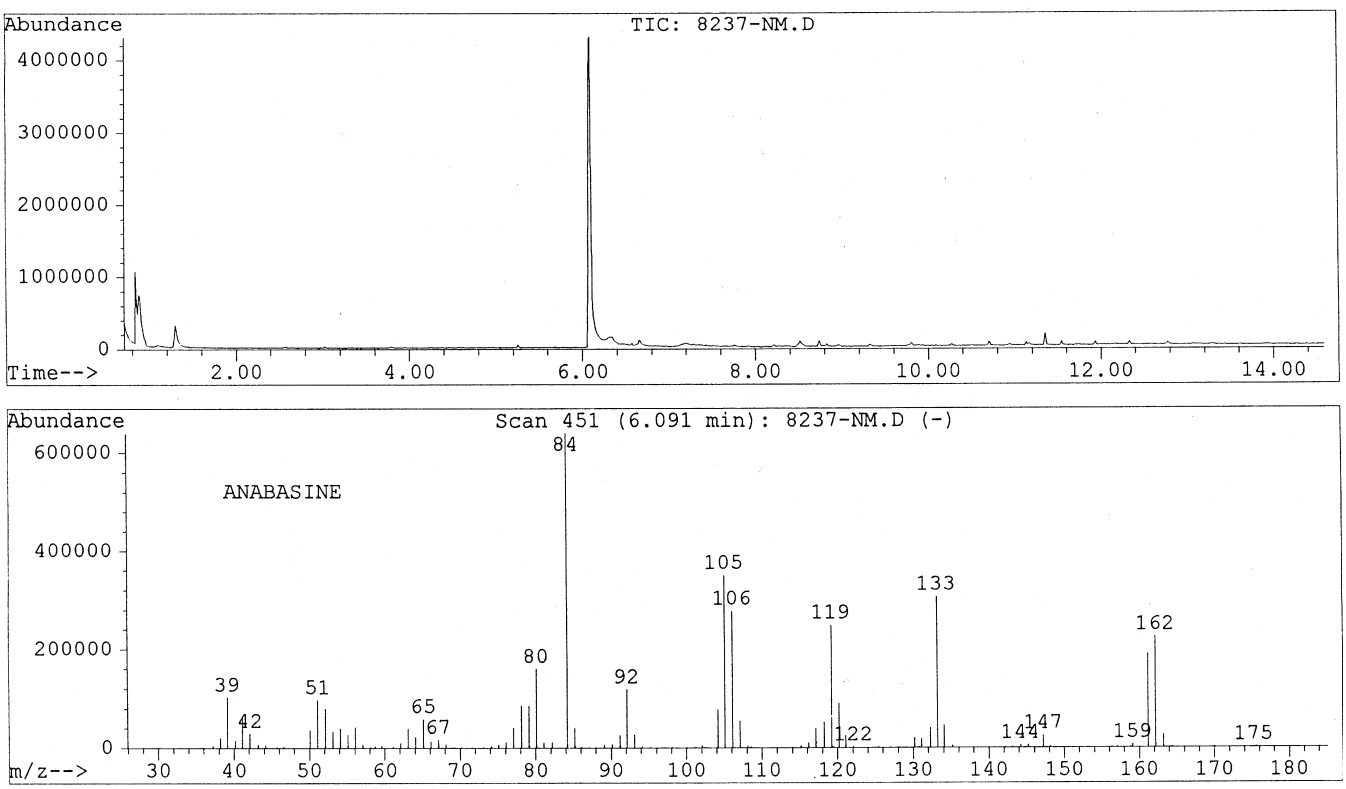

FIG. 3—*Mass chromatogram and mass spectrum of Nicotiana glauca extract.*

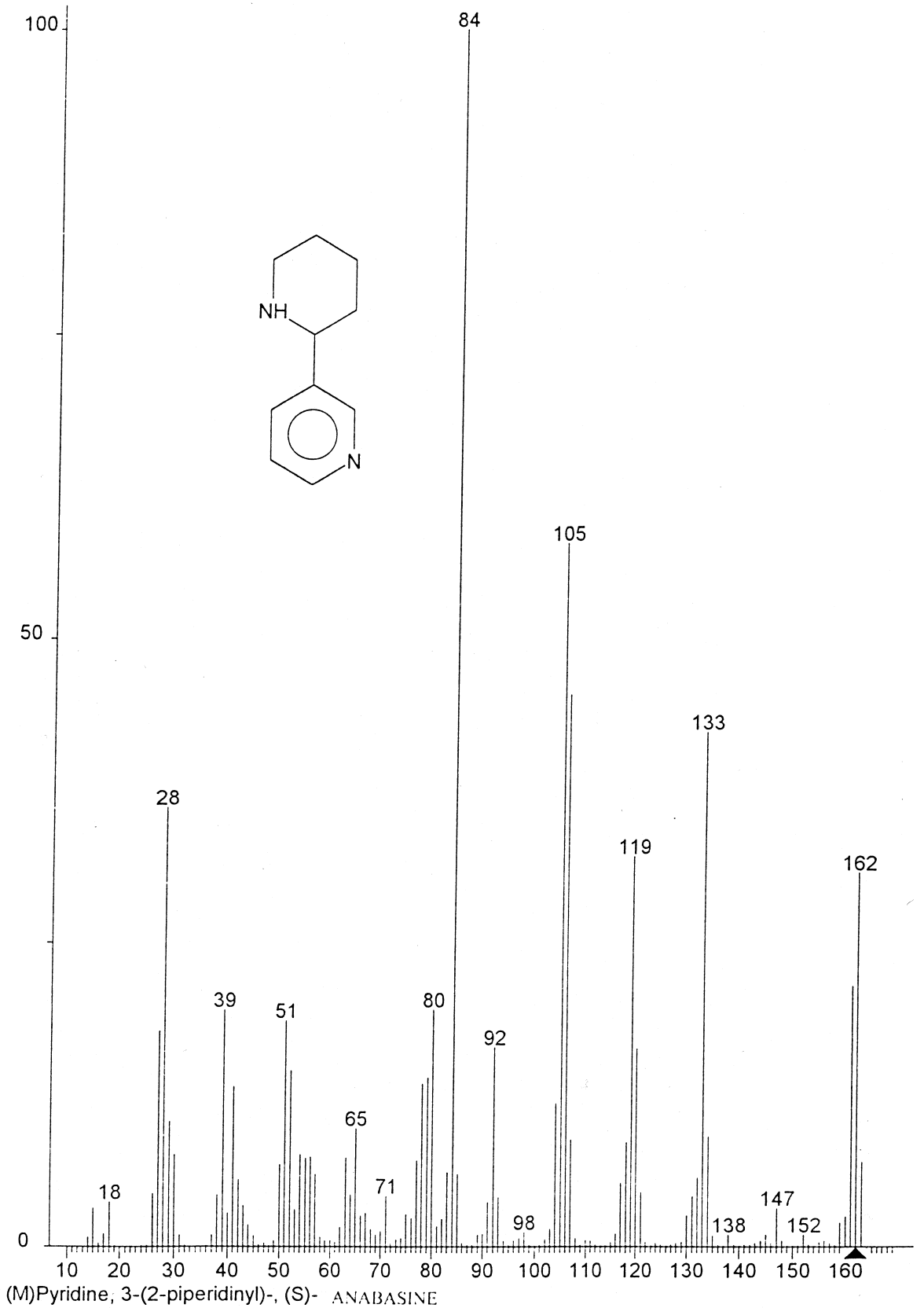

FIG. 4—*Mass spectrum of anabasine.*

Summary

Since the two patients ate the plant Nicotiana glauca, and any lack of other significant findings at an autopsy of the victim, the identification of anabasine in the urine and blood of the victim, it seems clear that the death of the late patient in case B and the symptoms of the patient from case A were direct results of the ingestion of leaves from Nicotiana glauca and the consequent anabasine poisoning. Cases of Nicotiana glauca ingestion and intoxication are common in both humans and animals, although the mortality to such incidents remains low. Perhaps the most reasonable option available for preventing Nicotiana glauca poisoning in animals would involve physically separating animals from the plant.

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