

CASE REPORT

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GC/MS Comparison of the West Indian Aphrodisiac "Love Stone" to the Chinese Medication "Chan Su": Bufotenine and Related Bufadienolides

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ABSTRACT: The death of a 23-year-old man resulting from digoxin-like toxicity and heart failure was attributed to ingestion of a West Indian aphrodisiac known as "Love Stone." GC/MS analyses identified bufotenine, a controlled substance under both US and New York State statutes. In addition, a series of bufadienolides, namely resibufogenin, bufalin, and cinobufagin, were also identified. Bufadienolides, which are derived from toad venom or secretions, are cardiotoxic steroids that cause symptoms similar to digoxin. GC/MS analyses of the Chinese medication "Chan Su," a product derived from toads, produced a highly similar elution profile and contained the same compounds as "Love Stone." The data demonstrate that the aphrodisiac was also derived from toads.

KEYWORDS: forensic science, Love Stone, Chan Su, GC/MS, bufotenine, bufadienolides, resibufogenin, bufalin, cinobufagin, street drugs, chemistry

The New York City Department of Health, Poison Control Center (NYCPCC), recently submitted a sample of a West Indian aphrodisiac "Love Stone" to our laboratory that was associated with the death of a 23-year-old man. The cause of death was digoxin-like toxicity and heart failure, which was attributed to ingestion of the aphrodisiac (1); this is the fourth such death from ingestion of "Love Stone" reported by the NYCPCC. The product is available in local gift shops, tobacco stores, and adult entertainment stores under a variety of names, including "Love Stone," "Black Stone," "Stud 100," "Hard Rock," and "Chinese Love Stone." It is intended to be moistened and applied externally to the penis to prolong erection. It is sold in unlabeled clear plastic heat-sealed packets that are sometimes arrayed on cardboard posters displaying pictures of exotic women. Some (but not all) packets are marked:

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"For External Use Only." Each packet contains a small reddish brown irregular shaped cube (ca. $\frac{1}{2}$ by $\frac{1}{2}$ by 1 in.) that has a resinous texture. The average piece varies in mass from 0.5 to 1.5 g.

Capillary column GC/MS analyses of the aphrodisiac identified bufotenine (an isomer of psilocin), a controlled substance under US and New York State statutes. In 1992, the New York City Police Laboratory (NYCPL) identified bufotenine in a product they originally suspected was "hashish" (2). The physical description of the product reported by the NYCPL (2,3) was identical to "Love Stone," and microscopic examination of both products revealed no plant-like material.

The Chinese medicine "Chan Su," which is derived from toads, is also known to contain bufotenine and a series of bufadienolides (4). Bufadienolides are cardiotoxic steroids that produce physiological symptoms similar to digoxin and have also been shown to be cross-reacting compounds in digoxin immunoassay analyses (5). Capillary column and megabore column GC/MS analyses (with on-column injection) produced virtually identical elution profiles for both "Love Stone" and "Chan Su." In addition to bufotenine, a number of compounds including the bufadienolides resibufogenin, bufalin, and cinobufagin, were identified in both products. The data demonstrate that the aphrodisiac was also derived from toads, rather than from the originally suspected plant material.

"Love Stone" is also used as a hallucinogen, as recently reported in an extensive review by Chamakura (3). Because the identification of bufotenine in 1992 by the NYCPL (2), other forensic laboratories in Tampa, Florida (6), Philadelphia, Pennsylvania (7) and Suffolk County, New York (8), have reported similar findings. In addition, articles describing the collection of toad secretions, and how it is dried and smoked for hallucinogenic purposes, have appeared in *Scientific American* (9) and local newspapers (10-12).

This report describes the GC/MS comparative analyses and identification of bufotenine and bufadienolides in the aphrodisiac "Love Stone" and the Chinese medicine "Chan Su."

Experimental

Capillary Column GC/MS Analyses

Capillary GC/MS analyses were performed using a Finnigan 9611 GC directly interfaced to a Finnigan TSQ-45 mass spectrome-

ter. Data were acquired with a SuperIncos^R data system. The GC was equipped with either (a) 30 m by 0.32 mm inside diameter, 0.25- μ DB-17 fused silica capillary column, temperature programmed from 50°C (3-min hold) to 320°C at 20°C/min, or (b) 15 m by 0.25 mm inside diameter, 0.25- μ DB-1 fused capillary column, temperature programmed from 100°C (2-min hold) to 290°C at 20°C/min (10-min hold). In both cases, the injector and transfer lines were 250°C, the injector was operated in the splitless mode, and Helium was used as the carrier gas at 12 psi. The mass spectrometer was operated in the electron impact (EI) mode, at 70-eV ionization energy, 500- μ A emission current. The ionizer and manifold temperatures were 130 and 90°C, respectively. The scan range was m/z 33 to 650 in 0.5 s.

Megabore on-Column Injection GC/MS Analyses

Megabore on-column GC/MS analyses were performed using a Varian 3400 GC interfaced by means of a glass jet separator to a Finnigan TSQ-700 MS. Data were acquired with a ICIS data system. The GC was equipped with a 15 m by 0.53 mm inside diameter, 0.25- μ DB-1 megabore column, temperature programmed from 200°C (1-min hold) to 280°C at 20°C/min. On-column injection was used, with a Helium flow rate of 30 mL/min. The injector and transfer lines were 250 and 270°C, respectively. The mass spectrometer was operated in the EI mode, 70-eV ionization energy, 500- μ A emission current. The ionizer and manifold temperatures were 150 and 80°C, respectively. The scan range was m/z 33 to 500 in 2 s.

Materials

Bufotenine, psilocin, and psilocybin were obtained from the US Drug Enforcement Administration's Northeast Regional Laboratory, New York, NY. Bufalin, cinobufagin, cinobufotalin, cholesterol, campesterol, and β -sistosterol were obtained from Sigma Chemical Co., St. Louis, MO.

Results and Discussion

Since the initial death report by the NYCPC indicated digoxin-like poisoning, the aphrodisiac was first analyzed for digoxin. Direct exposure probe mass spectral (MS) analyses using EI, methane CI, and CI/MS/MS techniques were all performed. Neither digoxin or digoxigenin was detected at a screening level of 0.01%.

A methanol extract of the aphrodisiac was then analyzed by GC/MS using a 30 m DB-17 capillary column to determine if other drugs were present. MS data indicated the major component had a molecular weight of 204 with a base peak at m/z 58. The relative abundance of the few fragment ions observed were all less than 20%. A library search produced good matches for three hallucinogens: psilocin (4-hydroxy-N,N-dimethyltryptamine), psilocybin (the phosphate ester of psilocin), and bufotenine (5-hydroxy-N,N-dimethyltryptamine), plus one nonhallucinogenic product, caulophylline (N-methylcystisine). Except for psilocybin (MW = 284), the compounds all have the same molecular weight (204) and molecular formula, C₁₂H₁₆N₂O. Positive ion CI/MS (methane) confirmed a molecular weight of 204. To rule out caulophylline (the only candidate containing no exchangeable protons),

a deuteration study was performed. The sample extract was evaporated to dryness, two drops of D₂O were added, and the residue redissolved in acetone and rerun on the GC/MS. The molecular ion shifted up two mass units, thereby ruling out caulophylline.

Reference standards of psilocin, psilocybin, and bufotenine were then subjected to GC/MS analysis on the same system. The MS data for all three compounds (Fig. 1) are very similar; all three display a significant molecular ion [M]⁺ at m/z 204 and a base peak at m/z 58. The only differences in the spectra of bufotenine compared with psilocin and psilocybin are slight variances in the relative abundance ratios of the m/z 130, 146, 117, and 118 ions. However, the GC retention time (R_t) of bufotenine, #2 (15:43) is sufficiently different from those of psilocin, #1 (15:33) and psilocybin, #1a (15:33) to confirm its identity. The R_t of bufotenine reference material was also identical to that of the sample. Similar MS and R_t data for bufotenine, psilocin, and psilocybin have been reported by Vohlken (6) and Chamakura (2).

GC/MS analysis of a locally obtained sample of "Chan Su" confirmed the presence of bufotenine. The relative levels of bufotenine in the "Love Stone" samples (consumer death and follow up sample) and "Chan Su" were 1.2, 0.3, and 0.8 %, respectively.

GC/MS analyses on a 15-m DB-1 capillary column were then performed to detect any additional compounds that may not have eluted off the 30-m DB-17 column. The elution profiles of the West Indian aphrodisiac and Chinese medicine were virtually identical (Fig. 2), and exhibited a number of additional compounds compared with the 30 m DB-17 capillary column. The mass spectra of all the corresponding compounds, #2 to #10, in both products were identical (Fig(s). 3, 4), indicating that both products were derived from the same or highly similar sources. The mass spectra of these compounds, #2 to #10, also agreed with the mass spectra reported by the NYCPL (3).

In an attempt to detect the bufadienolides that did not elute off the 15-m DB-1 capillary column, on-column injection onto a 15-m DB-1 megabore column was performed. This technique resulted in detection of the bufadienolides and some additional compounds. The GC/MS elution profiles of both products were again virtually identical (Fig. 5), and the mass spectra of the corresponding compounds were also identical (Fig(s). 6, 7). Cholesterol, #12, and campesterol (methyl cholesterol), #13, were identified by direct comparison of both the MS and R_t data of the sample compounds to the respective reference standards. β -Sistosterol (ethyl cholesterol), #14, was tentatively identified by comparison with reference grade material; however, the specific isomeric form was not confirmed. Two reports in the literature differ on the isomeric form of sistosterol present in "Chan Su"; Ruckstuhl et al. (13) identified the isomer as γ -Sistosterol, although Sakuria et al. (14) identified the isomer as β -sistosterol.

The same series of bufadienolides were observed in both the aphrodisiac and Chinese medicine. Resibufogenin, #15, [(3 β ,5 β ,15 β)-14,15-epoxy-3-hydroxy-5-bufa-20,22-dienolide] was tentatively identified by comparison with the NIST library. Bufalin, #16, [(3 β ,5 β ,14 β)-3,14-dihydroxy-5-bufa-20,22-dienolide], and cinobufagin, #17, [(3 β ,5 β ,15 β ,16 β)-14,15-epoxy-3,16-dihydroxy-5-bufa-20,22-dienolide-16-acetate], were identified by direct comparison of both the MS and R_t data with the respective reference standards.

The mass spectrum of #18, is similar to that of bufotalin, [(3 β ,5 β ,14 β ,16 β)-3,14,16-trihydroxy-5-bufa-20,22-dienolide-16-acetate]; however, no standard was available for direct comparison. Compound #11 also appears to be a bufa-type compound, because it displays the characteristic ions m/z 107, 215, and 233 (as in

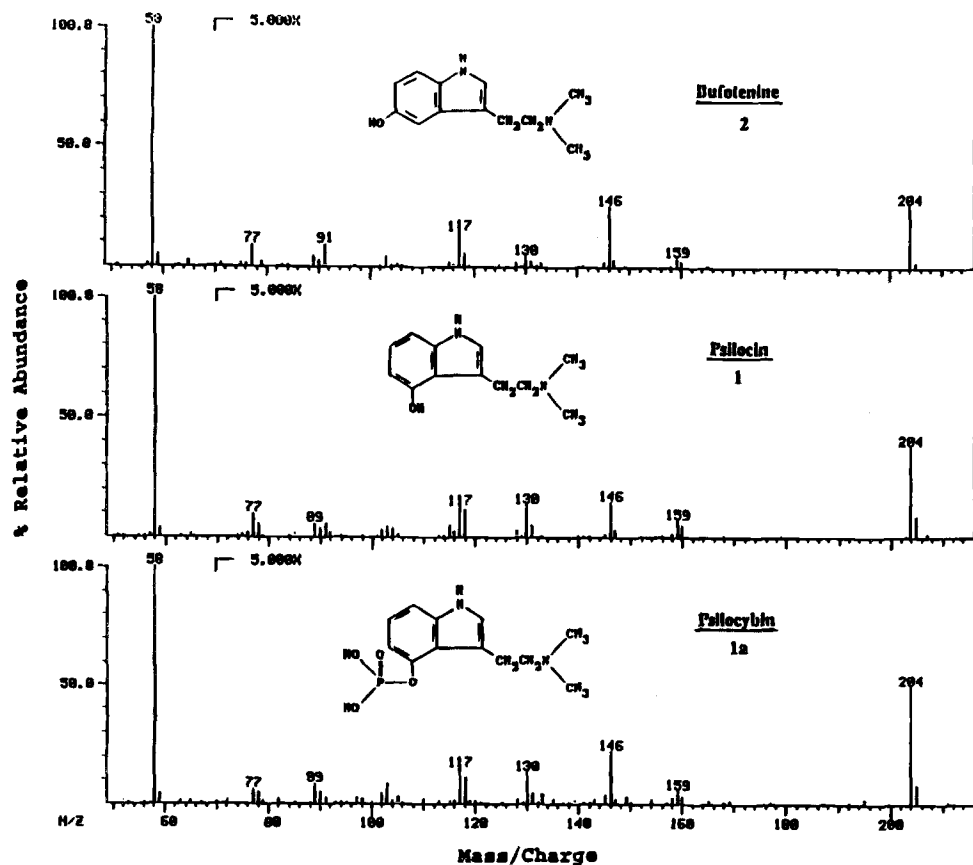


FIG. 1—Mass spectra of bufotenine, psilocin, and psilocybin.

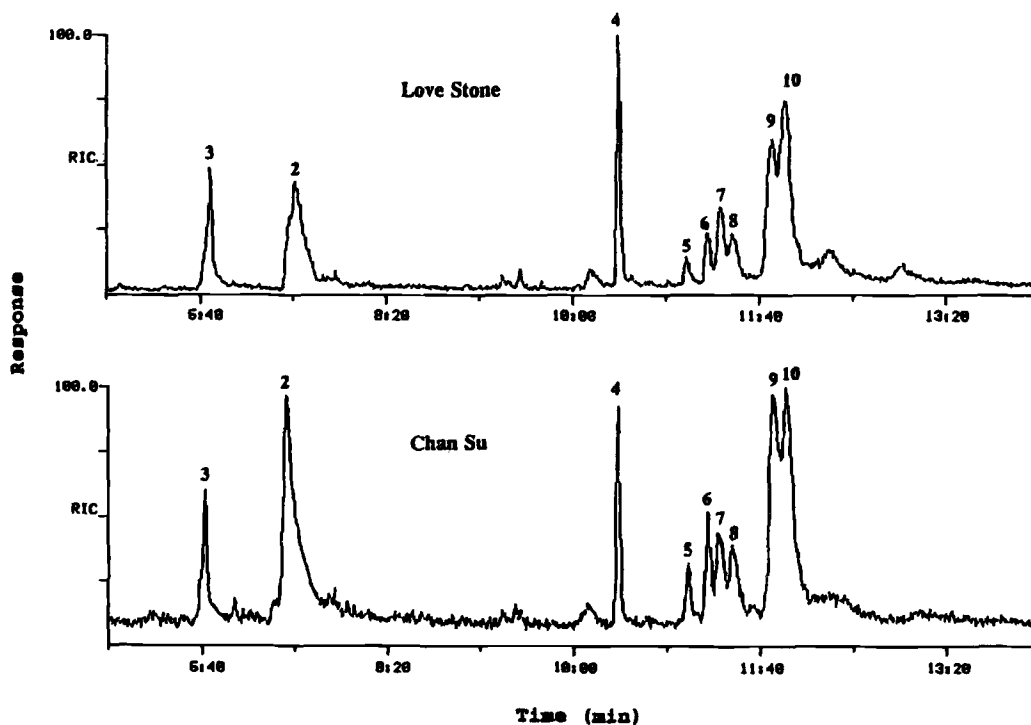


FIG. 2—15-m DB-1 capillary column GC/MS elution profile of the aphrodisiac "Love Stone" and Chinese medicine "Chan Su."

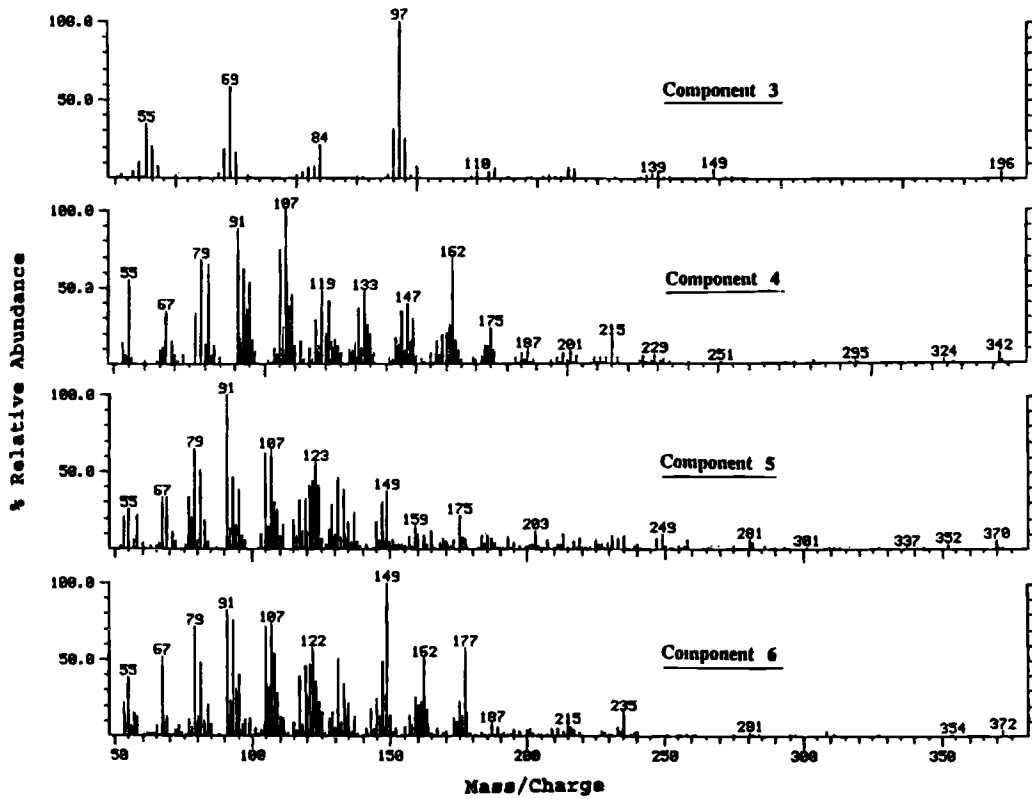


FIG. 3—Mass spectra of compounds #3 to #6 in “Love Stone” and “Chan Su.”

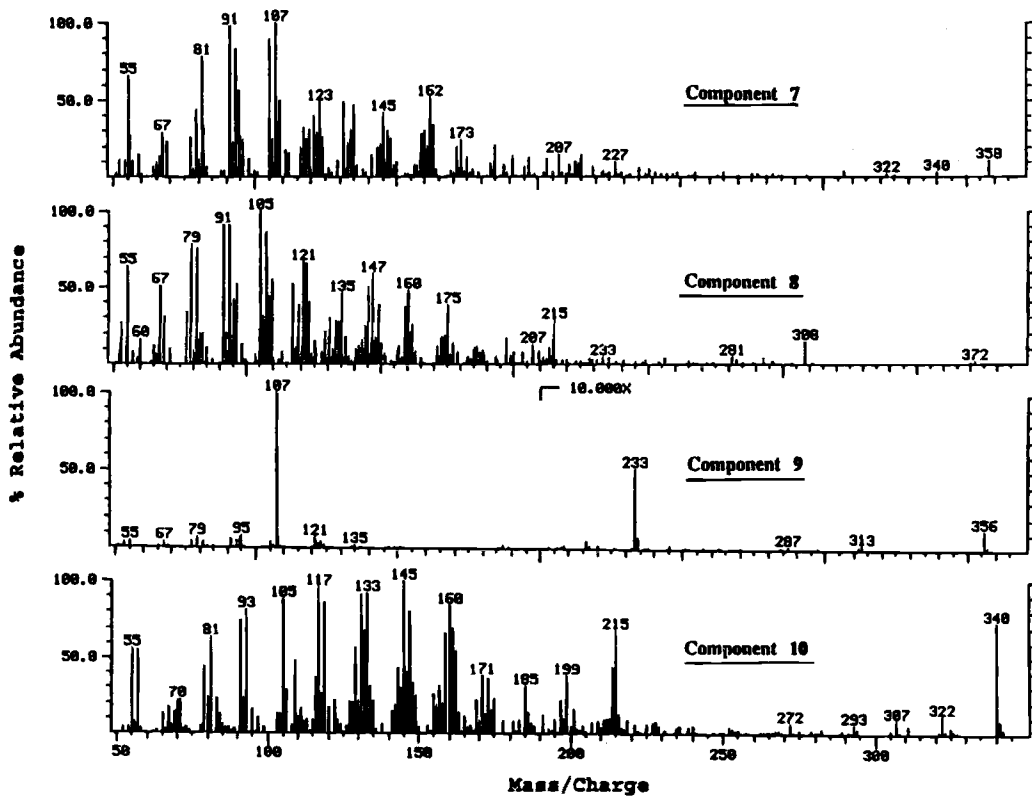


FIG. 4—Mass spectra of compounds #7 to #10 in “Love Stone” and “Chan Su.”

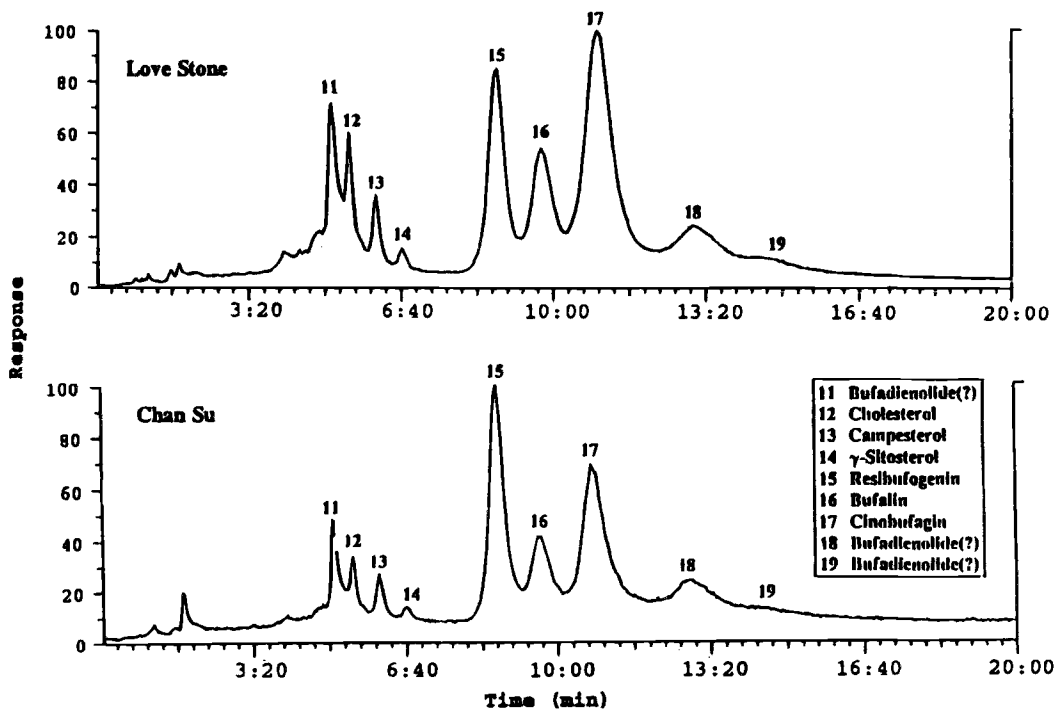


FIG. 5—15-m DB-1 Megabore (on-column injection) GC/MS elution profile of "Love Stone" and "Chan Su."

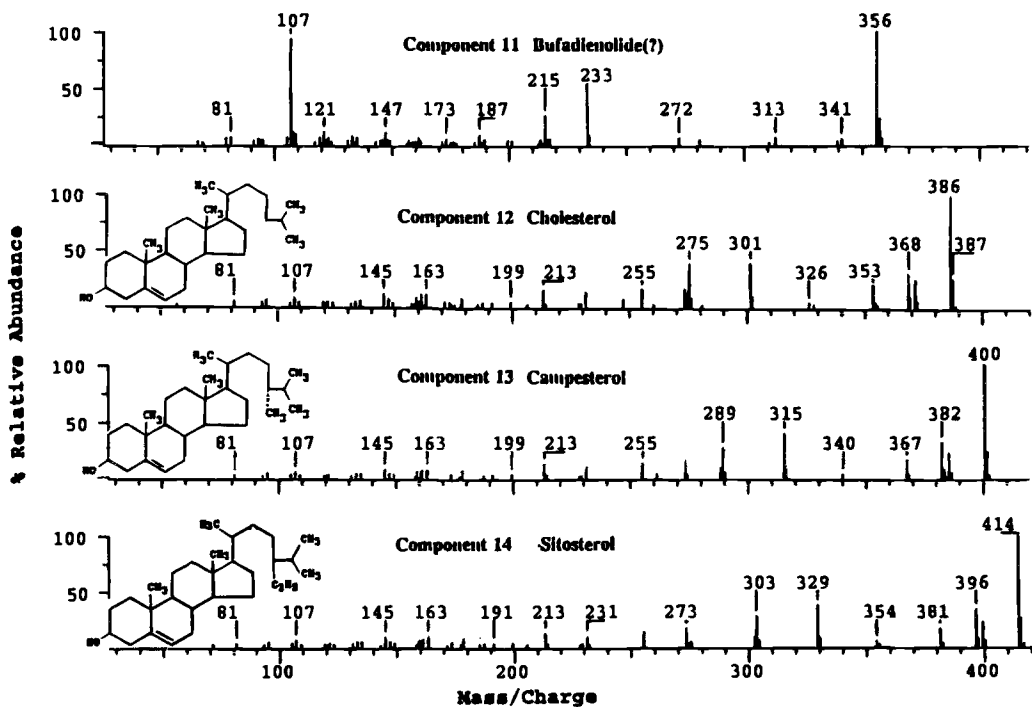


FIG. 6—Mass spectra of compounds #11 to #14 in "Love Stone" and "Chan Su."

resibufogenin and cinobufagin); again however, no identification was made. The mass spectral data of all the bufadienolides agreed with their previously reported low resolution solid probe mass spectra (15,16).

Conclusion

The West Indian aphrodisiac "Love Stone" and the Chinese medicine "Chan Su" displayed virtually identical GC/MS profiles

on three different columns, indicating that both products are derived from toads or another highly similar source. A series of bufadienolides, namely resibufogenin, bufalin, and cinobufagin, were identified in both products using a 15-m DB-1 megabore column with on-column injection. Bufotenine was also identified in both products. Bufotenine is currently being used as both an hallucinogen and an aphrodisiac, and is the latest recreational drug of abuse appearing in the New York City area. Ingestion of various aphrodisiac products

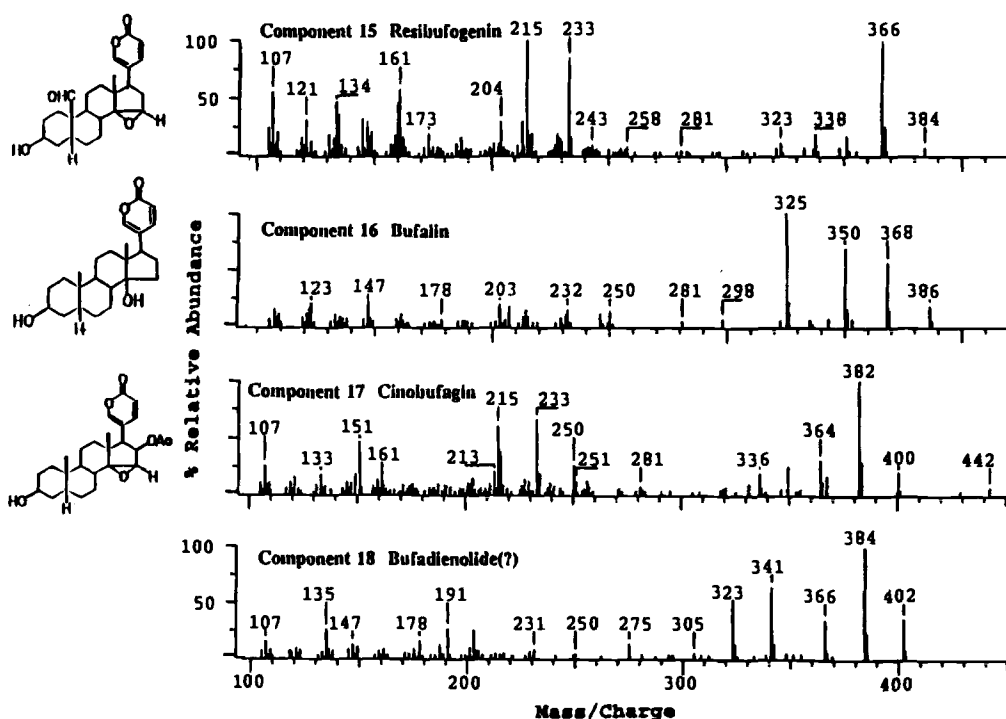


FIG. 7—Mass spectra of compounds #15 to #18 in "Love Stone" and "Chan Su."

containing bufotenine and bufadienolides have resulted in several deaths.

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