*R. M. Smith,*[1] *Ph.D.*

Forensic Identification of Opium by Computerized Gas Chromatography/Mass Spectrometry

Opium, the dried exudate of unripe capsules of the opium poppy (*Papaver somniferum* L.), is a complex mixture containing up to 25 different alkaloids in varying amounts [*1*]; yet it is a mixture whose identification is frequently demanded in forensic laboratories. Of the 25 alkaloids, only five are generally present in significant quantities in crude opium: morphine (Ia; about 10 percent); codeine (Ib), thebaine (II), papaverine (III), and narcotine (IV)—each present in amounts varying from 0.5 to 5 percent. At present forensic identification of suspected opium relies heavily upon general characteristics of the mixture, such as chemical spot tests, thin-layer chromatography, and gas chromatography. Obtaining good spectral data on the major alkaloids present in these mixtures has been precluded, however, due to the difficulty of isolating any of these compounds in reasonably pure form [*2*]. This difficulty can be overcome with combined gas chromatography/mass
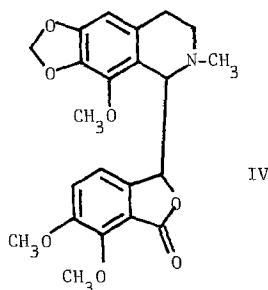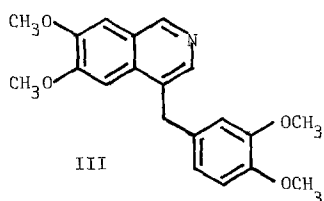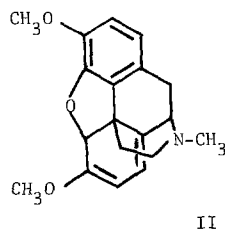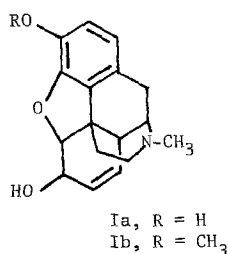
Ia, R = H
Ib, R = CH$_3$

II

III

IV

spectrometry (GC/MS), whose ability to produce spectral data from the constituents of small quantities of complex mixtures already has led to significant advances in the area of drug identification [3–7]. This paper describes a method using computerized GC/MS for the identification of suspected opium samples.

Experimental

Apparatus

The instrumentation used in this work consisted of a Hewlett-Packard 5930A modified quadrupole (dodecapole) mass spectrometer; a Hewlett-Packard 5700A gas chromatograph equipped with a 6 ft by 2 mm inside diameter glass column packed with 3 percent OV-101 on "high performance" Chromosorb W, 80–100 mesh, and interfaced to the mass spectrometer inlet system by a single stage silicone rubber membrane; and a Hewlett-Packard 5932A data system equipped with software for mass spectrometer control, data reduction, and mass spectral interpretation. The entire instrument package is commercially available as described. The temperature of the column, interface, and ionization source was 250°C; that of the GC injection port was somewhat higher. Helium, at a flow rate of 30 ml/min, was used as the carrier gas. The ionizing voltage was 70 eV.

Data were collected by the data system using a "SCAN" program, in which the major functions of the mass spectrometer were under computer control. The computer was instructed to collect a complete mass spectrum [50–400 atomic mass units (amu)] of the GC effluent every 4 s (scan speed—325 amu/s) and to store the data obtained on cassette tape. The length of the GC run was preset at 10–15 min, so that 150–225 spectra per sample were recorded. The computer scans were activated 1.0 min after injection of the sample to allow the solvent to pass through the spectrometer undetected. The data were reduced by a "CHROMATOGRAM RECONSTRUCT" program to give plots of total ion current per mass spectral pass versus scan number [$= \frac{1}{4}(t - 60)$, where t is the GC retention time in seconds]. Individual mass spectra from each run were also recalled for plotting and tabulation by appropriate programs. Spectral correlations were made both visually and with the aid of a "LIBRARY SEARCH" program, in which unknown spectra were compared to those in a library of 130 standard drug spectra. In every case high correlations were found between sample spectra and those of the appropriate drugs contained in the library.

Chemicals

Standard samples of powdered opium (designated sample #1), morphine, and codeine (Merck and Company, Rahway, N.J.) were dissolved in a small volume of 3:1 chloroform/methanol, as were two samples of confiscated illicit opium. One of the illicit samples (designated sample #2) was a dark, extremely hard, solid material, whereas the other illicit sample (sample #3) was a dark syrup mixed with water. Standard papaverine was prepared by basic extraction from a sample of Pavabid® (Marion Laboratories, Kansas City, Mo.). Concentrations of morphine in the opium solutions were presumed to be on the order of 5–10 mg/ml, assuming that morphine constitutes 10 percent of the dry weight of crude opium [1]. Concentrations of the other major alkaloids thus were presumed to be on the order of 1–4 mg/ml. For each run, 1 μl of solution was used for GC/MS analysis.

Thebaine was isolated from a chloroform/methanol solution of standard opium by preparative thin-layer chromatography (TLC) on silica gel using Davidow's solvent (ethyl acetate/methanol/aqueous ammonium hydroxide 85:10:5). The morphine alkaloids were located by spraying an edge of the developed plate with iodoplatinate solution;

three brown bands of significant intensity were observed at Rf values 0.69 ± 0.08, 0.47 ± 0.08, and 0.26 ± 0.08. All three bands were extracted from the silica gel with absolute ethanol and examined by direct probe mass spectrometry. The two bands having lower Rf values proved to be morphine and codeine; the mass spectrum of the uppermost band (Rf ≈ 0.69) was quite similar to that published for thebaine [8].
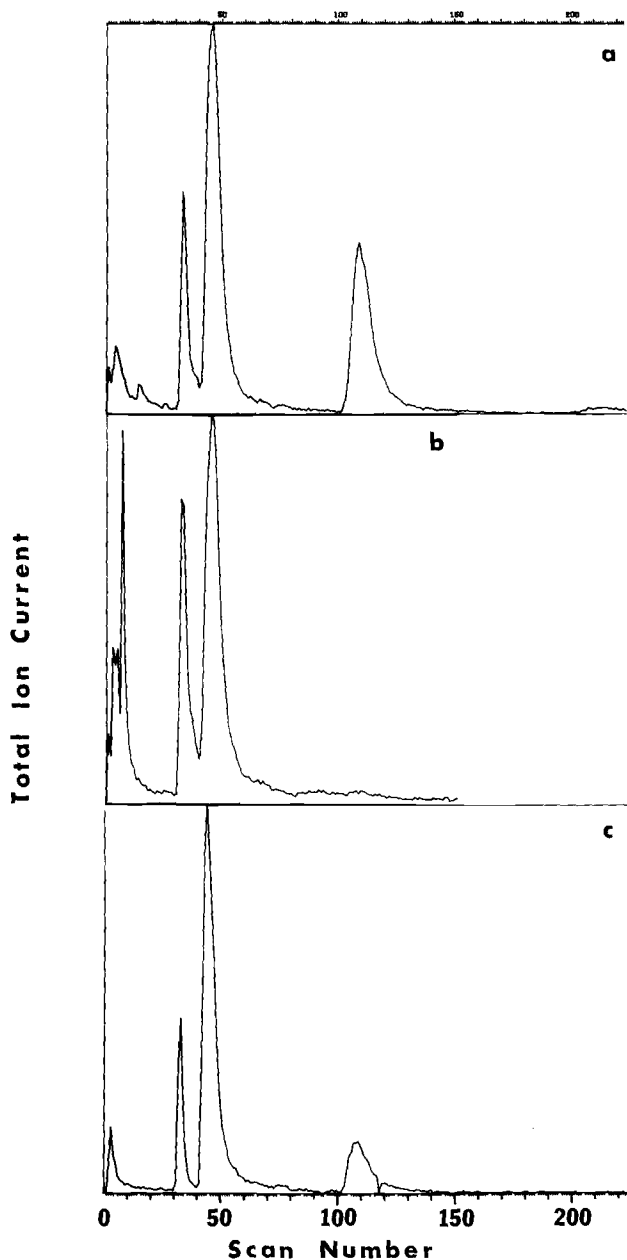

FIG. 1—*"Reconstructed chromatograms" of opium samples #1 (a), #2 (b), and #3 (c).*

Results and Discussion

Plots of total ion current versus scan number ("reconstructed chromatograms") for standard opium (sample #1) and the two illicit samples (samples #2 and #3) are shown in Fig. 1. Two peaks, occurring at scan numbers 33 ± 1 and 45 ± 1 are common to all three samples. The mass spectral data actually obtained in scans 33 and 44 from each of these samples are reproduced in Figs. 2 and 3, respectively.

In accord with prior suspicions, the compound giving rise to the chromatographic peak at scan 33 was identified as codeine because of the similarity between these spectra and that of standard codeine obtained under identical conditions. The spectra all exhibit an
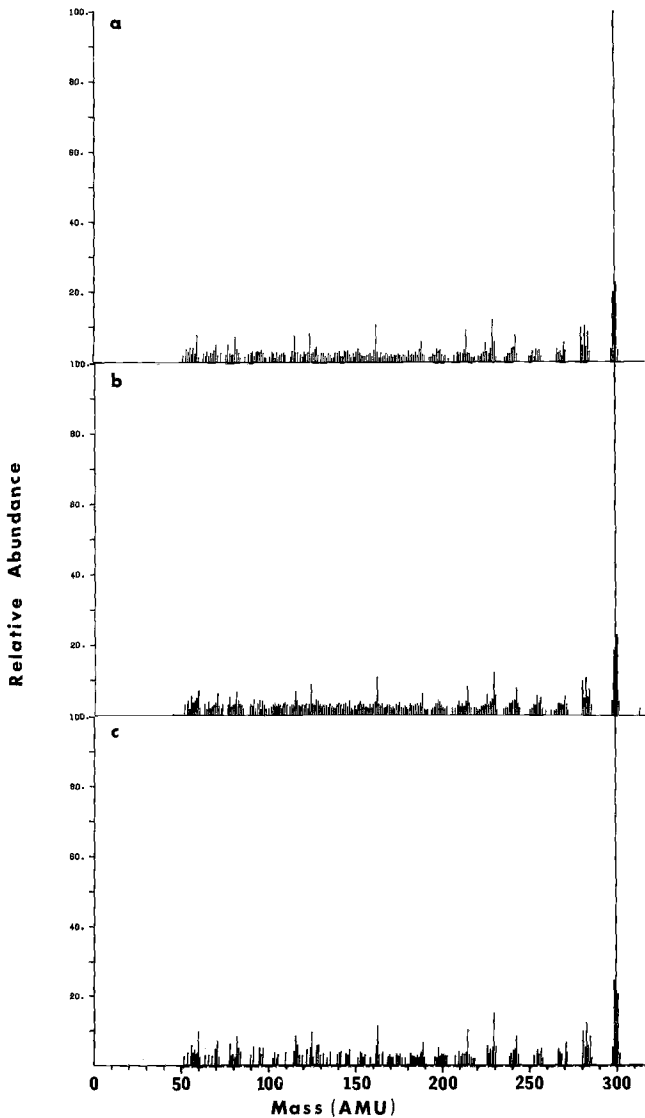

FIG. 2—*Mass spectra from scans 33 (codeine) of opium samples #1 (a), #2 (b), and #3 (c).*

intense molecular ion peak at mass to charge ratio (m/e) 299 and important fragment ions at m/e 229 and 162. These ions, which are highly indicative of the codeine structure, also occur in published spectra of codeine [8]. Similarly the mass spectra from scans 44, exhibiting molecular ion peaks at m/e 285 and important fragment ions at m/e 215 and 162, were identified as those of morphine after comparison with spectra of standard morphine obtained under identical conditions and by comparison with published spectra [8].

In addition to the two peaks at scans 33 and 45, a third smaller chromatographic peak occurs at scan number 109 in samples #1 and #3. This compound appears to be entirely
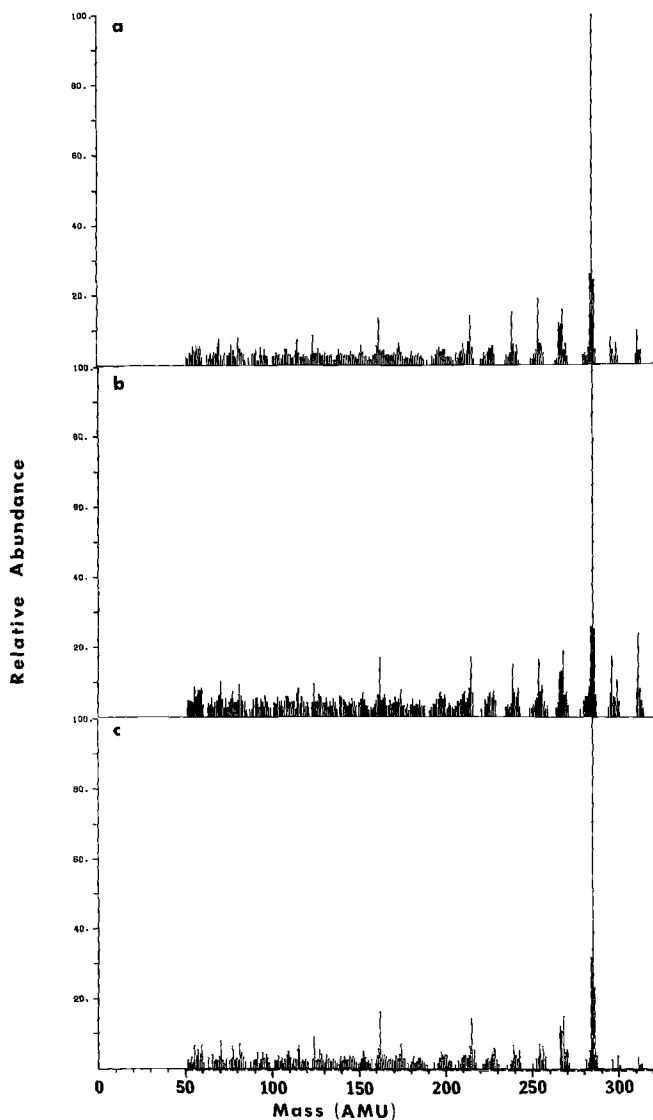

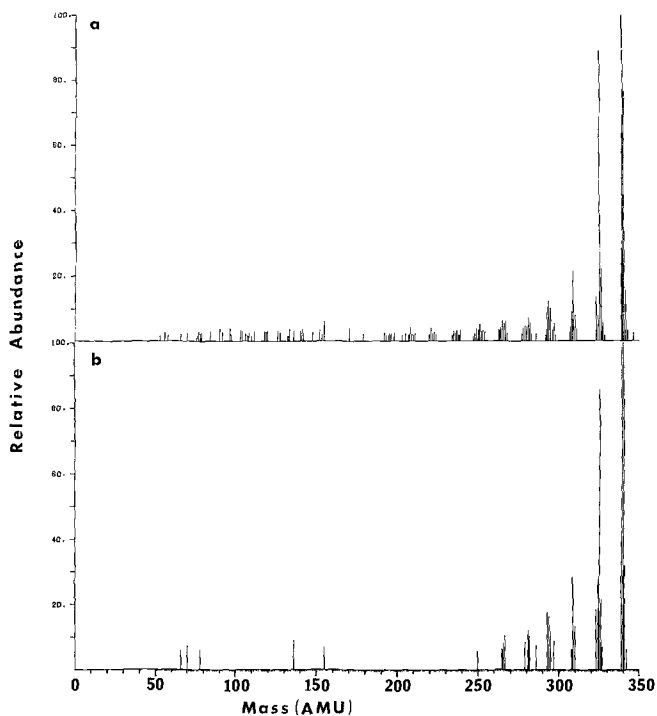FIG. 3—Mass spectra from scans 44 (morphine) of opium samples #1 (a), #2 (b), and #3 (c).

FIG. 4—*Mass spectra from scans 109 (papaverine) of opium samples #1 (a) and #3 (b).*

absent from sample #2. The mass spectra of this compound (Fig. 4) were very characteristic, showing intense peaks at m/e 339 (molecular ion, M⁺), 338 (M − 1), and 324 (M − 15). Of the five major alkaloids listed in structures I–IV, only papaverine (III), having a molecular weight of 339 and four ionizable methyl groups, further has a doubly benzylic hydrogen atom to account for the facile loss of a single hydrogen seen in the mass spectrum. Comparison of the spectra from these samples with that of standard papaverine run under similar conditions established the identity of this constituent as papaverine.

Establishing the presence of thebaine in the opiate mixture was not as straightforward as desired. In the first place, the GC retention time of thebaine under these conditions was only slightly longer that that of morphine, so that thebaine eluted as a tail on the morphine peak. That this tailing (seen most prominently in samples #1 and #2) was due to a compound other than morphine was evident from an examination of mass spectral scans 44–48 from these samples. In each case peaks of significant intensity (in scans 48, they were the most prominent peaks in the spectrum) were observed at m/e 311 (molecular ion for thebaine; see Figs. 3a and 3b) and m/e 296. Whereas these spectra lacked any sizable peak at m/e 255 (an obviously prominent peak in published spectra of thebaine [8]), the mass spectra of thebaine obtained from standard opium by preparative thin-layer chromatography and measured after sample introduction by gas chromatography were quite comparable to those obtained from samples #1 and #2 (Fig. 5a). On the other hand, mass spectra of the same purified material measured after direct sample introduction on a heated probe were not significantly different from literature spectra of thebaine [8] (Fig. 5b). This purified material had a GC retention time virtually identical with that
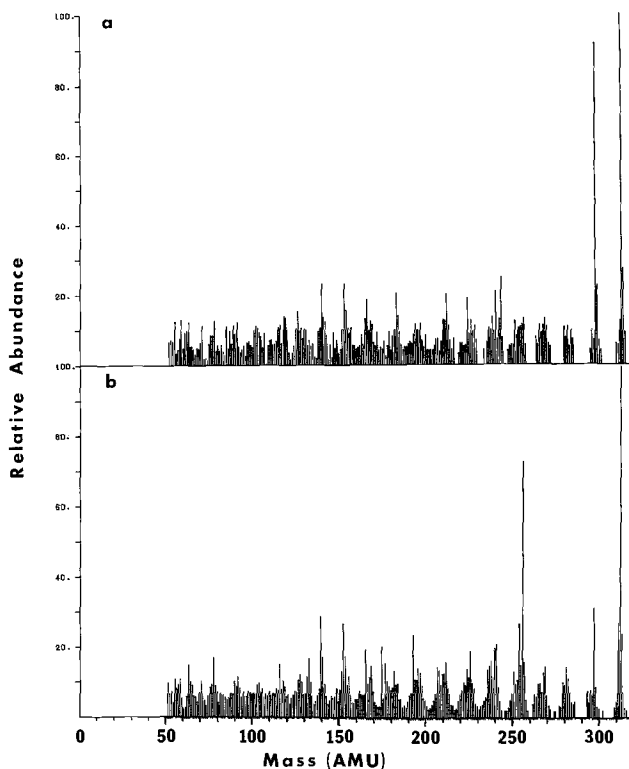
FIG. 5—*Mass spectra of standard thebaine obtained after sample introduction by* (a) *gas chromatography and* (b) *heated probe.*

of the materials in samples #1 and #2, and thus, it appears that thebaine may have undergone thermal rearrangement or degradation or both on the GC column. This was confirmed to a certain extent by the fact that the mass spectra of purified thebaine obtained after direct sample introduction were free of peaks due to morphine and codeine (m/e 285 and 299), while the "reconstructed chromatograms" of the same sample after sample introduction by GC showed significant amounts (10–20 percent) of these compounds to be present. It further seems unlikely that the purified thebaine was contaminated with morphine or codeine, since these compounds were easily excluded by the preparative TLC scheme (see **Experimental** section). Any products from these thermal reactions were not characterized further.

Narcotine (IV) was not detected in any of these samples under the conditions of these experiments. Mass spectra of the volatile materials observed in sample #2 (scan numbers 2–15) were examined, but identification of these compounds was not possible on the basis of these data.

Summary

Mass spectral data identifying the major alkaloids of opium were easily obtained by computer-monitored mass spectral analyses of the GC effluent from suspected opium samples. In particular, identifiable mass spectra of morphine, codeine, thebaine, and papaverine were obtained by GC/MS/computer analysis of a standard opium sample,

and the presence of three of these alkaloids in each of two illicit samples was demonstrated by their mass spectra.

Acknowledgments

References

[1] Hoover, J. E. (managing editor), *Remington's Pharmaceutical Sciences*, Mack, Easton, Pennsylvania, 1970, pp. 492–493, 1122–1123.
[2] Mathers, A. P. and Butler, W. P., *Methods of Analysis for Alkaloids, Opiates, Marihuana, Barbiturates, and Miscellaneous Drugs*, Internal Revenue Service Publication No. 341, 1967, pp. 45–76.
[3] Skinner, R. F., Gallaher, E. J., Knight, J. B., and Bonelli, E. J., "The Gas Chromatograph-Mass Spectrometer as a New and Important Tool in Forensic Toxicology," *Journal of Forensic Sciences*, Vol. 17, 1972, pp. 189–198.
[4] Nakamura, G. R., Noguchi, T. T., Jackson, D., and Banks, D., "Forensic Identification of Heroin in Illicit Preparations Using Integrated Gas Chromatography and Mass Spectrometry," *Analytical Chemistry*, Vol. 44, 1972, pp. 408–410.
[5] Law, N. C., Aandahl, V., Fales, H. M., and Milne, G. W. A., "Identification of Dangerous Drugs by Mass Spectrometry," *Clinica Chimica Acta*, Vol. 32, 1971, pp. 221–228.
[6] Hammar, C.-G., Holmstedt, B., Lindgren, J.-E., and Tham, R., "The Combination of Gas Chromatography and Mass Spectrometry in the Identification of Drugs and Metabolites," *Advances in Pharmacology and Chemotherapy*, Academic Press, New York, Vol. 7, 1969, pp. 53–89.
[7] Karasek, F. W., "GC/MS/Computers," *Analytical Chemistry*, Vol. 44, 1972, pp. 32A–42A.
[8] Wheeler, D. M. S., Kinstle, T. H., and Rinehart, K. L., "Mass Spectral Studies of Alkaloids Related to Morphine," *Journal of the American Chemical Society*, Vol. 89, 1967, pp. 4494–4501.

Wisconsin State Crime Laboratory
4706 University Avenue
Madison, Wisconsin 53705