Morphine and Codeine in Poppy Seed

Poppy seed (*Papaver somniferum* L.) was analyzed for free and bound morphine and codeine by gas chromatography. A commercial sample of blue seed contained 0.5 to 1.7 ppm of free morphine and 0.1 to 0.5 ppm of free codeine. Bound alkaloids liberated by acid hydrolysis from two sources of blue and one source of white seed ranged from 0.6 to 4.2 ppm of morphine and 0.5 to 1.5 ppm of codeine. The identity of the alkaloids was confirmed by gas chromatography–mass spectrometry of their trimethylsilyl ether derivatives.

Although several studies of alkaloids in seed of the opium poppy (*Papaver somniferum* L.) have been reported, the question of whether morphine is an actual seed constituent has not been settled (Duke, 1973). Mika (1955) found no morphine in a 5-g sample of poppy seed. Sarkany et al. (1970) reported several alkaloids but not morphine or codeine in seed coat, endosperm, and embryo fractions. Preininger et al. (1965) were unable to detect morphine in one sample until after they incubated the seed at 37 °C for 48 h. However, in another sample they found components corresponding to morphine, codeine, and thebaine by paper and thin-layer chromatography. Fairbairn and El-Masry (1968) detected bound codeine, plus unidentified alkaloids, in seed previously extracted to remove free alkaloids present in dust or adhering latex. These bound alkaloids were defined as those liberated from seed material by acid hydrolysis, pepsin digestion, or fermentation. They found a phenolic alkaloid chromatographically similar to but spectroscopically different from morphine.

In studying factors affecting the morphine content of *P. somniferum* capsules, we determined morphine by gas chromatographic (GC) analysis of the bis(trimethylsilyl) ether derivative (Tookey et al., 1975). We report here the application of this technique to the analysis of poppy seed for traces of morphine and codeine and the use of combined gas chromatography–mass spectrometry (GC–MS) to identify these alkaloids.

MATERIALS AND METHODS

Seed Sources. Blue B-40-6-3 and white M-89 seed of *Papaver somniferum* L. was obtained from experimental plots in Arizona, May 1974; commercially available blue seed came from McCormick and Co., Inc., Baltimore Md.

Extraction of Alkaloids. (A) Blue B-40-6-3 seed was sieved to remove a small amount of capsular debris. Samples of seed (100 g) were washed with acidified H_2O (pH 2, HCl, 3 × 130 ml) and once with distilled H_2O (100 ml). The water washes were freeze-dried, redissolved in H_2O (50 ml), and made alkaline to pH 8.5 with NH_4OH. This solution was extracted with $CHCl_3$–*i*-PrOH (3:1, v/v, 3 × 25 ml) to give surface alkaloids.

(B) Sieved and crushed seed (100 g) was extracted sequentially for 20-min periods by stirring on a steam bath with hexane (200 ml), EtOH (200 ml, 150 ml, 150 ml), and hexane (200 ml) to remove lipids and surface and free alkaloids. The combined extract was concentrated to dryness in vacuo, acidified with 0.5 N HCl (30 ml), and washed with hexane (3 × 50 ml). The aqueous phase was made alkaline to pH 8.5 with NH_4OH and extracted with $CHCl_3$–*i*-PrOH (3:1, v/v, 4 × 25 ml). The organic extract containing the alkaloids was concentrated and separated into nonphenolic and phenolic alkaloid fractions by solvent extraction acording to the procedure of Fairbairn and El-Masry (1968).

(C) In one experiment, hexane–EtOH extracted B-40-6-3 seed was stirred and refluxed in boiling H_2O for 2 h. The aqueous extract was freeze-dried and then refluxed in 2 N HCl for 2 h to liberate any bound alkaloids that may have been extracted by H_2O. Appropriate alkaloidal extracts were prepared from the concentrated hydrolysate (Fairbairn and El-Masry, 1968).

(D) To obtain bound alkaloid fractions, hexane–EtOH extracted air-dried seed material (average 67 g) was stirred and refluxed in 2 N HCl (250 ml) for 2 h. The hydrolysis mixture was cooled, diluted with EtOH (500 ml), and filtered through Celite with suction. The filtrate was concentrated in vacuo to 50–75 ml, made alkaline to pH 8.5 with NH_4OH, and extracted with $CHCl_3$–*i*-PrOH (3:1, v/v, 4 × 50 ml). The concentrated organic extract was separated into bound nonphenolic and phenolic alkaloid fractions (Fairbairn and El-Masry, 1968).

Table I. Morphine and Codeine Levels in *Papaver somniferum* L. Seed

Seed source	Extraction method[a]	Surface and free, ppm		Bound, ppm	
		Morphine	Codeine	Morphine	Codeine
B-40-6-3	A	15	3	*b*	*b*
B-40-6-3	A	15	3	*b*	*b*
B-40-6-3	B, C, D	*b*	*b*	4.2	1.5
M-89	B, D	13	1.5	2.6	0.6
Commercial	B, D	0.5	0.1	1.0	0.5
Commercial	B, D	1.5	0.5	*b*	*b*
Commercial	B, D	1.7	*c*	0.6	*c*
Commercial	B, D	*c*	*c*	1.7	*c*
Commercial	B, D	*c*	*c*	2.3	*c*

[a] Method A removes surface alkaloids; method B removes surface and any free internal alkaloids; method C would remove water-soluble bound alkaloids; however, none were detected; method D removes bound alkaloids. See Materials and Methods section for details. [b] Not analyzed. [c] Presence not confirmed by mass spectrometry.

GC Analysis. Alkaloid fractions were converted into Me_3Si derivatives and analyzed by GC as described previously (Tookey et al., 1975).

GC–MS. Effluent from the GC was directed to a Dupont (CEC) 21-492-1 (Dupont Instruments Corp.) mass spectrometer (MS) through a jet-type sample enricher. Spectra were obtained with an ionizing energy of 70 eV, a source temperature of 200 °C, a source pressure of 2×10^{-5} Torr, and a resolution of 1000. During each GC run, effluent was examined at a scanning rate of 4 s/decade from m/e 35 to 500. Scans were made repetitively at intervals of 8 s. Using computerized data reduction, plots were made of the total ionization current of each scan vs. scan number and of intensities of ions at m/e 429 and 371 vs. scan number, these ions being the molecular ions of $(Me_3Si)_2$ morphine and Me_3Si codeine, respectively. Mass spectra taken where maxima occurred in plots of m/e 429 or 371 vs. scan number verified the GC peak assignments.

RESULTS AND DISCUSSION

Alkaloid levels in two sources of blue and one source of white poppy seed are summarized in Table I. Morphine

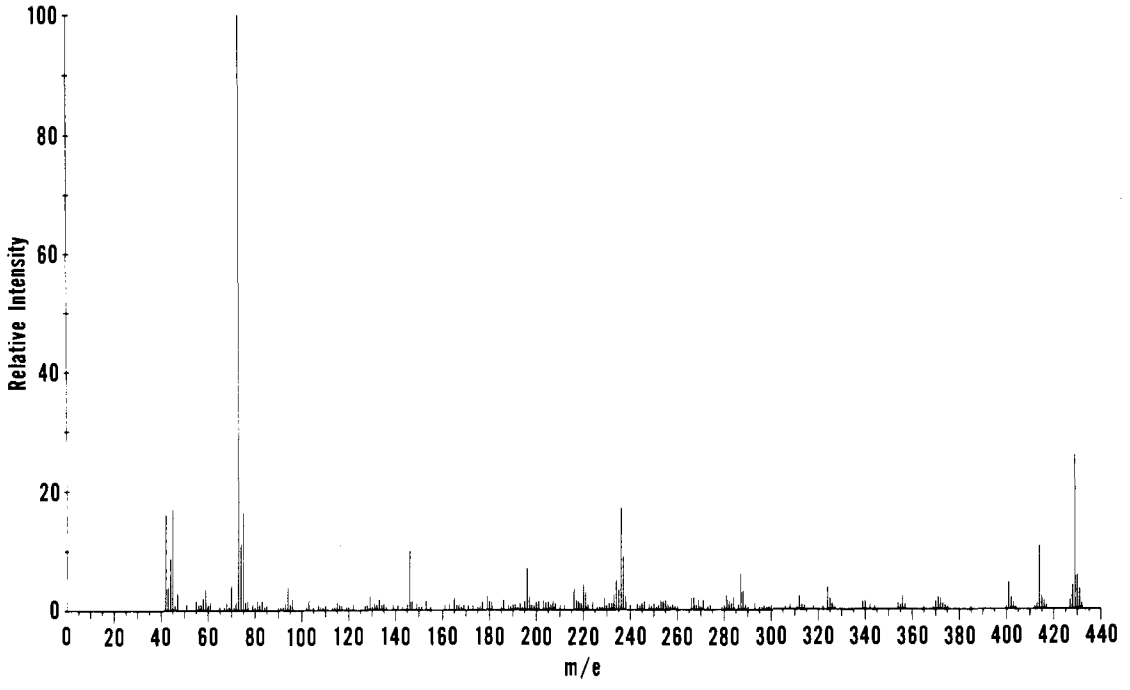

Figure 1. Mass spectrum of $(Me_3Si)_2$ ether derivative of bound morphine from poppy seed.

and codeine concentrations of 13 to 15 ppm and 1.5 to 3 ppm, respectively, in alkaloid fractions prepared from the Arizona seed by extraction method A or B are probably due to dust and dried latex adhering to the seed coat. Analysis of comparable fractions from commercial seed gave considerably lower values of 0.5 to 1.7 ppm of morphine and 0.1 to 0.5 ppm of codeine. These values obtained by extraction method B represent both surface and any free internal alkaloids.

Prior to analysis for bound alkaloids in B-40-6-3 seed, the crushed and hexane–EtOH-extracted seed material was extracted with boiling H_2O. The aqueous extract was hydrolyzed with HCl and alkaloidal extracts were prepared from the hydrolysate. Because neither morphine nor codeine could be detected by GC analysis, any bound alkaloids present in the seed were not in a water-soluble form.

When the residual seed material remaining after water extraction was hydrolyzed with 2 N HCl and after appropriate bound alkaloid extracts were prepared, 4.2 ppm of morphine and 1.5 ppm of codeine were found. The identity of $(Me_3Si)_2$ morphine under the GC peak was confirmed by its MS (Figure 1) which showed the same fragmentation pattern as that of a $(Me_3Si)_2$ morphine standard. Codeine Me_3Si ether was also confirmed by its MS, which exhibited a strong molecular ion at m/e 371. One sample of white M-89 seed contained comparable levels of 2.6 ppm of morphine and 0.6 ppm of codeine. Bound morphine in four replicate samples of commercial poppy seed ranged from 0.6 to 2.3 ppm. Bound codeine at 0.5 ppm was verified in only one sample. Values for both free and bound alkaloids should be considered maximal since the presence of interfering materials under the GC peaks cannot be ruled out. Carbohydrates extracted by EtOH or aqueous acid upon conversion to Me_3Si derivatives exhibit a GC peak very close to $(Me_3Si)_2$ morphine. However, typical carbohydrate fragment ions were absent from MS of the alkaloid peaks. Trace amounts of morphine and codeine in poppy seed do not constitute a potential threat for abuse. Assuming total bioavailability of both free and bound morphine, a person would have to consume approximately 3.5 kg of seed to obtain a therapeutic dose.

LITERATURE CITED

Duke, J. A., *Econ. Bot.* **27**, 390 (1973).
Fairbairn, J. W., El-Masry, S., *Phytochemistry* **7**, 181 (1968).
Mika, E. S., *Bot. Gaz.* **116**, 323 (1955).
Preininger, V. L., Vrublovsky, P., Stastny, V. L., *Pharmazie* **20**, 439 (1965).
Sarkany, S., Michels-Nyomarkay, K., Verzar-Petri, G., *Pharmazie* **25**, 625 (1970).
Tookey, H. L., Spencer, G. F., Grove, M. D., Duke, J. A., *Bull. Narc.* **27**(4), 49 (1975).

Michael D. Grove*
Gayland F. Spencer
Marjorie V. Wakeman
Harvey L. Tookey

Northern Regional Research Laboratory
Agricultural Research Service
U.S. Department of Agriculture
Peoria, Illinois 61604

Received for review November 19, 1975. Accepted April 26, 1976. The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.