Quantitation of the Major Alkaloids in Opium from *Papaver Setigerum* DC

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ABSTRACT: Quantitation of morphine and other major alkaloids in opium gum from specially cultivated *Papaver setigerum* DC ("Wild Poppy") is presented. *Papaver setigerum* plants (n = 14) were grown in an atmosphere containing a slightly elevated level of carbon dioxide (390 ppm). Opium gum collected from the capsules of the mature plants was analyzed for morphine, codeine, thebaine, noscapine, and papaverine, using capillary electrophoresis (CE). Morphine was confirmed at an average of 2 percent by weight. Codeine, noscapine, and papaverine were also detected; however, thebaine was below the limits of quantitation by the employed CE method, and could only be detected by gas chromatography/mass spectrometry.

KEYWORDS: *Papaver setigerum*, *Papaver somniferum*, Opium Poppy, Wild Poppy, Opium, Opium Alkaloids, Quantitation, Forensic Chemistry

Introduction

There are more than one hundred species under the genus *Papaver* that produce alkaloids in the specialized cells called laticifers. However, only two naturally occurring species, *Papaver somniferum* L (Photo 1, next page) and *Papaver setigerum* DC (Photo 2, next page) produce morphine in significant quantities [1]. *Papaver somniferum* is commonly known as opium poppy, and is cultivated around the world for both licit and illicit purposes. *Papaver setigerum*, also known as "wild poppy," is native to the Mediterranean region and Canary Islands. Although several previous reports indicate that *Papaver setigerum* opium contains morphine (*vide infra*), to date it has never been reported to have been used for licit or illicit morphine production.

Scientific publications on *Papaver setigerum* reveal varying opinions amongst scientists in labeling it as a separate species or as the subspecies of *Papaver somniferum*. One of the earliest publications on opium poppies from Fulton [2] suggested a close relationship between *Papaver somniferum* and *Papaver setigerum*. Farmilo *et al.* [3] was the first to report the presence of morphine in the pods, buds, and leaves of *Papaver setigerum*.

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Photo 1. Typical *Papaver Somniferum* Plant. Note the Horizontal Score Marks on the Capsules.

Photo 2. *Papaver Setigerum* Plant Grown for this Study. Note the Smaller, Elongated Capsules (Compare with Photo 1).

Farmilo *et al.* also indicated that the *Papaver setigerum* chromosome count is a tetraploid (n = 22), whereas *Papaver somniferum* is a diploid (n = 11), which suggests that *Papaver somniferum* could not have evolved from *Papaver setigerum* as many scientists had previously suggested. Farmilo's article also compared and contrasted sketches of *Papaver somniferum* and *Papaver setigerum* plants, including drawings of their pods (which differ significantly in appearance). The information supported earlier work by Sugiura [4], who disagreed with the concept of *Papaver setigerum* as a direct ancestor to *Papaver somniferum*, and instead regarded them as separate species, with the possibility of a common ancestor.

One of the earliest (1956) studies on the quantitation of morphine in *Papaver setigerum* opium came from Asahina *et al.*, who reported a morphine content of 5.1% [5]. A detailed publication from the same group in 1957 [6] described two sets of experiments with *Papaver setigerum* plants, reporting morphine values of 5.1% and 7.3% (surprisingly high). The other alkaloids were reported at: Codeine (0.9 and 0.8%); thebaine (2.1 and 1.6%); papaverine (1.9 and 2.6%); and narcotine (also known as noscapine, 0.1 and 0.1%). The paper also reported the relative differences in the alkaloids present in opium from *Papaver somniferum* cultivations in Iran, India, and Turkey versus those present in *Papaver setigerum* opium. A later study from La Valva *et al.* [7] found no morphine, codeine, and thebaine in the *Papaver setigerum* populations from Mediterranean France and southern Italy; however, this latter finding is atypical. Subsequently, Garnock-Jones *et al.* [8] confirmed alkaloids in the air-dried capsules of New Zealand cultivations of *Papaver setigerum* (morphine 0.4%; codeine 0.5%; papaverine 1.6%; narcotine 1.3%; thebaine not reported), but also noted that the alkaloid concentrations were "much lower" in wild plants. Garnock-Jones *et al.* also declared *Papaver setigerum* to be a subspecies of *Papaver somniferum*. Aside from the negative alkaloid values reported by La Valva *et al.*, these reports collectively established *Papaver setigerum* as a separate species (or possibly a rather distantly related subspecies), under the genus *Papaver*, with the potential of producing minor to significant amounts of morphine.

In the era of numerous internet vendors for seeds of both *Papaver somniferum* and *Papaver setigerum*, the forensic science community has a salient interest in the actual alkaloid composition of *Papaver setigerum*, and in the potential use of its opium for illicit purposes. As detailed above, the literature is inconsistent and in some cases is contradictory. The U.S. Controlled Substances Act does not differentiate between species (or subspecies) of *Papaver*. Thus, *Papaver setigerum* is not formally controlled (by name) in the United States; however, opium and related products (such as poppy straw) from *Papaver setigerum* are controlled (Schedule II) if they contain morphine, codeine, and/or thebaine. Of note, opium cultivation laws in many other countries specifically prohibit cultivation of *Papaver setigerum*.

The opium analyzed in this study came from an environmental research project conducted by the U.S. Department of Agriculture, where the primary goal was to study the effects of a slight increase in atmospheric carbon dioxide (CO_2) on *Papaver setigerum* plant growth and alkaloid production [9]. The Mauna Loa Observatory (Hawaii) reports the average ambient atmospheric CO_2 level to be 385 ppm [10]. However, the actual ambient atmospheric CO_2 level is thought to be slightly higher, as was reported by Ziska *et al.* [11]. Therefore, the study was conducted at a 390 ppm CO_2 level, which is believed to provide more valid data on actual alkaloid production in the wild. Fourteen *Papaver setigerum* plants were cultivated in controlled environment grow chambers in an atmosphere containing 390 ppm CO_2. Opium gum was obtained by lancing the mature pods from the plants, in the same manner that opium from *Papaver somniferum* plants is obtained, and was analyzed using a capillary electrophoresis methodology [12].

Experimental

Seeds: Seeds of *Papaver setigerum* DC were obtained from the Institut fur Pflanzengenetik und Kulturpflanzenforschung in Gatersleben, Germany.

Cultivation and Harvesting: The study was conducted using controlled environment chambers (EGC Corporation, Chagrin Falls, OH), with the chamber set at 390 ppm CO_2 for 24 h day-1. The actual average 24 h CO_2 values were 389 +/- 12.1 ppm. The seeds were sown by hand in 2.6 L pots filled with a 4:1:1 mixture of

sphagnum, perlite, and vermiculite. Floral initiation occurred at about 70 days after sowing. There were approximately 8-10 capsules per plant. The scoring of the mature capsules began about two weeks after the loss of the floral petals. Scoring was done using a razor blade, making 2 to 3 one-millimeter deep incisions on the capsule surface. For each capsule, opium gum was collected over a 24 h period on aluminum foil, allowed to air dry for 72 hours, and then weighed. The timing and harvesting techniques match those typically used for *Papaver somniferum*.

Capillary Electrophoresis (CE): Opium alkaloid standards were obtained from the reference collection of the DEA Special Testing and Research Laboratory (Dulles, VA). All CE-grade reagents and run buffer solutions were obtained from Microsolv™ Technology (Eatontown, NJ). High Performance Liquid Chromatography grade methanol was obtained from Burdick and Jackson (Muskegon, MI). High purity, deionized water was obtained from a Millipore Milli-Q-Gradient A10 water system (Bedford, MA). An internal standard stock solution of tetracaine hydrochloride was prepared by weighing 25 mg into a 100 mL volumetric flask and diluted to volume with a 1:11 mixture of methanol and 3.75 mM phosphate buffer (pH 3.2). To obtain the tetracaine internal standard working solution, 6 mL of the tetracaine HCl internal standard stock solution was diluted to volume with 3.75 mM phosphate buffer (pH 3.2) in a 50 mL volumetric flask. Appropriate amounts of morphine, codeine, thebaine, noscapine, and papaverine base standards were weighed into a 100 mL volumetric flask in order to obtain an approximate final concentration of 0.025 mg mL^{-1} for each compound. Ten mL of the internal standard stock solution was pipetted into the above mentioned volumetric flask and diluted to volume with a 1:11 mixture of methanol and 3.75 mM phosphate buffer. Approximately 500 µL of the solution was filtered using 0.45 µm regenerated cellulose Titan filter and transferred to a 1.0 mL polypropylene CE injection vial.

Appropriate amounts of the opium gum samples were weighed into a volumetric flask in order to obtain a concentration of morphine similar to that of the standard. The flask was filled to half volume with methanol and sonicated for 30 minutes at 55^0C to completely extract the alkaloids. The flask was then cooled, and diluted to volume with 3.75 mM phosphate buffer (pH 3.2). 400 µL of the above solution was added to 2.0 mL of the internal standard working solution. Approximately 500 µL of the above solution was filtered using a 0.45 µm regenerated cellulose Titan filter and transferred to a 1.0 mL polypropylene CE injection vial. An Agilent Model HP3DCE capillary electrophoresis system equipped with a diode array detector (Waldbronn, Germany) was used for alkaloid analysis and quantitation, as described by Lurie *et al.* [12]. All experiments were carried out with fused silica 32 cm (23.5 cm to detector window) x 50 µm I.D. pre-made capillaries obtained from Agilent Technologies (Part No: G1600-63211).

Gas Chromatography/Mass Spectrometry (GC/MS): Analyses were conducted using an Agilent (Palo Alto, CA) Model 5973 Quadrupole Mass Selective Detector (MSD) interfaced with an Agilent Model 6890 Gas Chromatograph (GC). The GC contained a J&W Scientific (Rancho Cordova, CA) 30 m x 0.25 mm I.D. fused silica capillary column coated with a film thickness of 0.25 µm DB-1. The injection port was maintained at 280^0C. The oven was programmed with an initial temperature of 90^0C, holding for 2 minutes, then 14^0C per minute increase until 300^0C, holding for another 10 minutes. One mL portions of the methanol extracts of the opium samples were placed into autosampler vials for analysis.

Results and Discussion

Morphine, codeine, noscapine, and papaverine were all detected and quantitated using the CE method described in the Experimental section. Thebaine was detected in the opium using the GC/MS method described in the Experimental section; however, CE quantitation of thebaine was not possible because of its low levels (the TIC indicated thebaine between 0.01 and 0.3%). The low thebaine content was unexpected in view of the value previously reported by Asahina *et al.* [6]. The quantitation results for morphine, codeine, noscapine, and papaverine for the opium samples collected from all 14 plants are presented in Table 1. The amounts of opium obtained from each plant are also presented in Table 1. The average levels were 2% morphine, 3% codeine, 10% noscapine, and 5% papaverine, all by weight of opium. The noscapine quant was stunningly high (5x) relative to

the morphine content, and interestingly, is very similar (on a weight percent basis) to the noscapine content of opium from *Papaver somniferum* cultivations in South and Central America [12].

Studies at the Special Testing and Research Laboratory have shown that the morphine content in *Papaver somniferum* opium varies among growing regions. However, on average, *Papaver somniferum* opium contains about 10 - 13% morphine [13]. These latter results do not derive from controlled cultivations; however, as noted above, it is assumed that these plants were grown at an average ground level CO_2 level of 390 ppm. The electropherograms of typical *Papaver setigerum* and *Papaver somniferum* opiums are shown in Figure 1.

Based on the results of this study, although *Papaver setigerum* opium contains some morphine, it is not a viable source of opium for illicit poppy cultivators. Cultivation and the manual harvesting of opium poppy capsules by lancing and subsequent hand-collection are very time-consuming and labor-intensive processes. *Papaver setigerum* plants are much smaller in size compared to *Papaver somniferum* plants; furthermore, the capsules in *Papaver setigerum* are also very small and elongated in shape (between 10 - 16 millimeters in length and between 4 - 7 millimeters in diameter). In contrast, *Papaver somniferum* capsules are more or less globular in shape and much larger in size (typically between 20 and 40 millimeters in diameter). For these reasons, the total amount of opium that can be obtained from a field of *Papaver setigerum* poppies is much lower than from an equal sized field of *Papaver somniferum* poppies. The much lower morphine content, much lower total opium yield, and the increased time and labor needed to harvest the opium from the small, elongated pods, all explain why *Papaver setigerum* has never received much attention in the areas of either licit or illicit cultivation and morphine production. Based on this study, it would appear that this disinterest is justified.

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Table 1. Alkaloid Composition of *Papaver setigerum* Opium
(Note: Thebaine was Detected, not Quantified).

Plant Number	Weight of opium obtained from each plant (mg)	Morphine%	Codeine%	Papaverine%	Noscapine%
1	38.1	2.5	3.0	5.2	11.4
2	24.1	2.4	2.9	5.0	10.9
3	38.8	2.3	2.7	4.7	10.3
4	27.6	2.5	2.2	4.8	9.8
5	35.2	3.1	1.8	5.1	10.4
6	34.2	2.1	2.9	5.4	10.8
7	82.6	2.3	2.0	5.5	11.2
8	52.3	2.4	3.0	4.4	9.8
9	67.9	2.3	2.1	5.5	10.5
10	23.1	1.4	3.5	4.6	10.9
11	27.6	2.5	2.6	4.2	9.6
12	21.4	2.0	3.2	4.9	10.1
13	16.9	2.8	2.7	3.3	8.5
14	19.8	1.7	2.4	3.8	9.0
Average	**36.4**	**2.3**	**2.6**	**4.7**	**10.2**
Std Dev	19.132	0.427	0.495	0.660	0.832
Min		1.4	1.8	3.3	8.5
Max		3.1	3.5	5.5	11.4

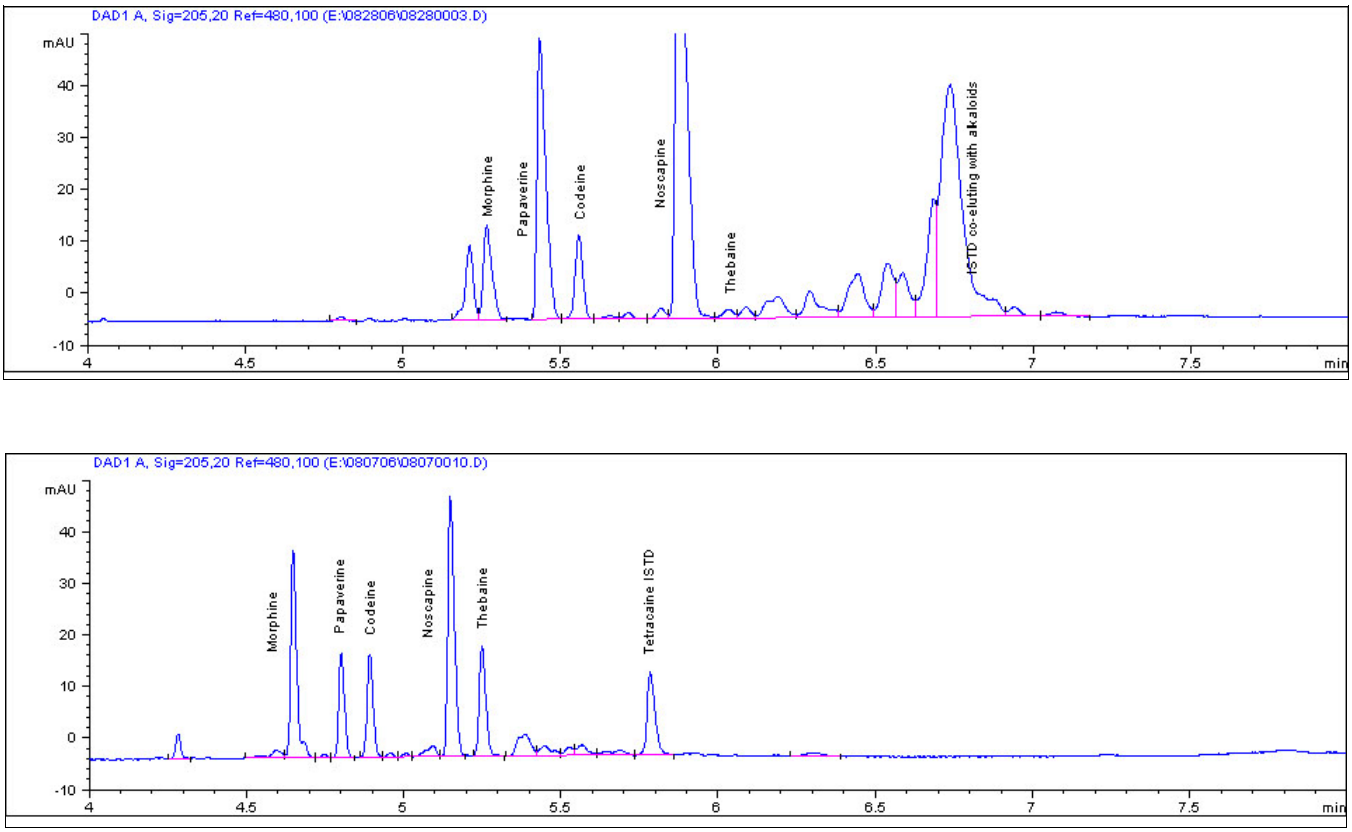

Figure 1. Electropherograms of Typical *Papaver setigerum* (Top Trace) and *Papaver somniferum* (Bottom Trace) Opiums. Time (x-axis) Differs for the Two Runs Because They Were Completed on Distantly Removed Dates (the older the capillary, the longer the retention times; however, the retention order and relative retention times are consistent). The *Papaver somniferum* Opium Electropherogram Presents Data from Opium Poppies Recently Grown in Afghanistan.

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