

ALKALOIDS IN THE GERMINATING SEEDLING OF POPPY

FRANK A. CRANE AND JAMES W. FAIRBAIRN

College of Pharmacy, University of Illinois at the Medical Center, Chicago, and School of Pharmacy, University of London

ABSTRACT.—Seedlings of the commercial "blue" strain of *Papaver somniferum* L. were germinated in culture dishes on wet gauze. Eleven gram or larger samples (f.wt.) were killed on successive days, extracted and chromatographed on silica gel G plates (0.25 mm.) in the following system: xylene, ethylmethylketone, methanol and diethylamine (20:20:3:1). Color development was with Dragendorff's reagent.

By the 3rd day of germination traces of morphine, codeine, thebaine, papaverine and narcotine could be detected. On the 5th day traces of possible reticuline could be seen, and narcotoline was suspected on the 6th day. Though all of the compounds increased in the extracts with increasing days up to the 9th day, the largest spots were thebaine, papaverine and narcotine. Difference in alkaloid composition between seedling and capsule was largely that of concentration. The seedlings contained much lower concentrations of all alkaloids observed.

Attempts were made to study development of these compounds by feeding C¹⁴ sucrose and tyrosine at the beginning of germination, but incorporation into these alkaloids was so slight that results were not conclusive.

The alkaloids of poppy (*Papaver somniferum* L.) are located in the laticifers of the plant. Identification and study of these compounds generally involves isolation from the vegetative parts (root, stem, leaves) and especially the developing capsules. It is commonly believed that poppy seeds do not contain alkaloid compounds, probably related to the fact that laticifers have not developed in the embryo of the mature

seed. By far the greatest amount of latex and alkaloid content is present in the pericarp and placenta during the period in which fertilized ovules are developing into seeds. Speculation on this condition has led to the idea that substances non-alkaloidal in nature must be passed from the placenta to the developing seeds. Alcoholic extracts of poppy seeds do not contain any substance which reacts with Dragendorff's reagent.

Germinated seedlings contain alkaloids beginning at the 3rd day after water is applied to the seeds. Though the seedling is very small (average weight per seed 0.54 mg.; average dimensions of seed 0.96 x 1.5 x 0.5 mm.) large numbers of seedlings of a single strain may be pooled for experimental work since development is very uniform. Many studies have been made of the alkaloidal composition of the mature plant (Mary, N. Y. and Brochmann-Hanssen, 1963; Genest, K. and Farmilo, 1962) while very few have attempted to characterize the alkaloids of the seedling (Massicot, J. 1961)

In this present work we have re-examined the changing alkaloidal picture during seedling development and attempted to discover whether the alkaloids are synthesized from

glucose or tyrosine in these early stages of growth and to relate our results to work on the possible importance of alkaloids to seed viability.

MATERIALS AND METHODS

Seeds of the commercial Blue Strain of poppy were soaked in 1-10 hypochlorite-water for 10 minutes and washed with 5 changes of distilled water. Four grams of seed were evenly spread on the water moistened surface of cotton gauze on the bottom of a deep 6" diameter culture dish. The glass cover was set in place and the dishes were located on a laboratory windowsill so that they would receive sunlight. Covers were removed periodically to allow air change, and water was added by pipette to keep the gauze moist but avoid pools of water. Sunlight for short periods was desirable, but caused condensation which was harmful if dripped directly on the seedlings. Dishes were observed and adjusted several times daily. Emergence of the root began by 48 hours after water was added, and a root system was observed by 3 days. Cotyledons could be seen at 4 days. Germination proceeded until the cotyledons were 7 cm. above the root at 9-10 days.

At harvest, the seedlings were immersed in a killing bath of 5 ml. 5% acetic acid in 100 ml. 70% methanol. Care was taken to remove roots from the gauze with fine forceps. Maceration in the fixing bath continued for 48 hours after which seedlings were triturated in a mortar and pestle or ground in a blender, then filtered through a Büchner funnel combin-

ing all washings. The solution was made alkaline to pH 8, with dry powdered sodium bicarbonate in a separatory funnel. It was then extracted with 4 to 5 successive 30 ml. portions of ethyl acetate. The combined ethyl acetate extract was evaporated to dryness at reduced pressure and the methanol-water extract was also evaporated.

An alternative system used for extraction of seedlings included the following. Seedlings were killed in 95% ethanol, macerated, ground and filtered. The pH was adjusted to acid range with 0.1 N hydrochloric acid and the solution was extracted with several portions of ethyl acetate to remove pigments and lipids. Ammonium hydroxide was added to bring the pH above 7.6 and 5 successive extractions were made using chloroform 3 parts to isopropanol 1 part, the aliquots 30, 20, 20, 20, 10 ml. These fractions were combined, were washed with water, then evaporated to dryness under reduced pressure. The water-soluble extract was also reduced to dryness. Each of the dried extracts were taken up in 2.0 ml. of methanol and held for chromatographic separation.

Extracts were chromatographed on thin layer plates of silica gel G (250 microns) spread by the DeSaga apparatus and activated at 105° for 30 minutes. One-tenth to 0.2 of the 2 ml. of extract (representing about 1000 seedlings) chromatographed satisfactorily.

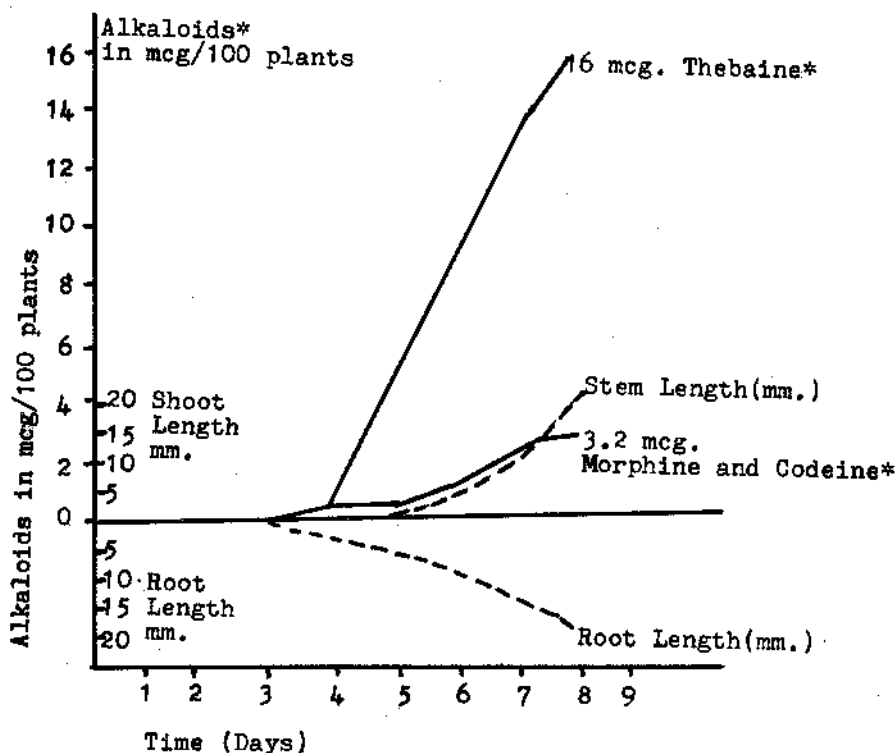
Plates were developed in the following solvent system in a saturated atmosphere of xylene, ethylmethylketone, methanol, diethylamine (20:20:3:1). Development occurred in 35-40 minutes. The solvent front

was marked immediately upon removal of the plate from the developing tank.

Color was revealed by use of the modified Dragendorff's solution which was sprayed lightly over the entire plate, followed by mild heating. Alkaloids appeared as orange-red spots against a yellow background. Concentrations required that certain alkaloids receive a second spraying with Dragendorff's reagent. Spots were circled immediately after development.

RESULTS AND DISCUSSION

Seeds were originally soaked in dilute hypochlorite solution and rinsed to eliminate molds which hinder germination. During the 24 hours the seeds had swollen to approximately double their original size. On the second day of germination emergence of the root occurred as enlargement of the germinating seed continued. Root length was about 2 cm. on the 3rd day and the cotyledons had begun to emerge from



* Alkaloid concentrations as reported by Massicot 1961.

FIGURE 1. Growth of seedlings and production of alkaloids during the course of germination of poppy (*Papaver somniferum* L.).

the seed coat. Fourth day seedlings bore roots of 3-4 cm. length and shoots of 5.6 mm. On the 5th day the shoot stood erect, about 2 cm. in height; the root was 5-6 cm. in length. The 6th day seedlings had cotyledons well expanded, were green in color, and were about 3-4 cm. tall. Roots were 6-8 cm. in length and had grown intertwined through the gauze substrate. Seventh, 8th and 9th days saw seedlings continue to the extent of their growth in the dishes. Maximum shoot length was around 5 cm. and maximum root length was 8 cm. At no time did the primary growing

point give rise to leaves beyond the stage of expanded cotyledons.

The presence of alkaloids in the seedlings followed the pattern shown in Figure 1.

No Dragendorff reactive material was found present in ground seeds of Blue Strain poppy regardless of the amount of seed used. This was also true after 24 hours of germination. On the 2nd day of germination the extract from a very large number of seedlings contained substances which reacted with the Dragendorff reagent but these were not resolved as discrete spots. Several samples of this extract were chromatograph-

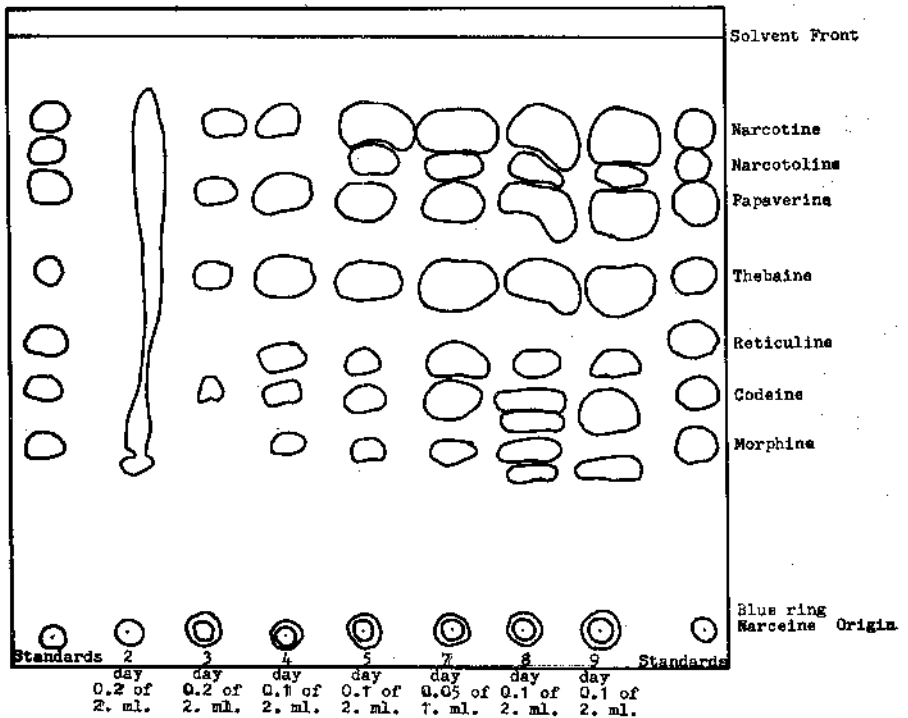


FIGURE 2. Composite of thin layer chromatograms of extracted seedlings of poppy (*Papaver somniferum* L.).

ed, but the streaks were not resolved.

Third day seedlings contained small but definite amounts of codeine, thebaine, papaverine, and narcotine with traces of presumably narcotoline and reticuline. Narceine, a water-soluble alkaloid, was also present in trace amount at the original point of application on the chromatogram (Figs. 2 and 3).

Extracts from 4th day seedlings produced larger, more definite spots of thebaine, papaverine, narcotine, narcotoline and reticuline, and in addition had small amounts of codeine and morphine present.

Fifth day seedlings contained all of the alkaloids previously mentioned in larger amounts for the amount of extract used to prepare the plates was half of that for the

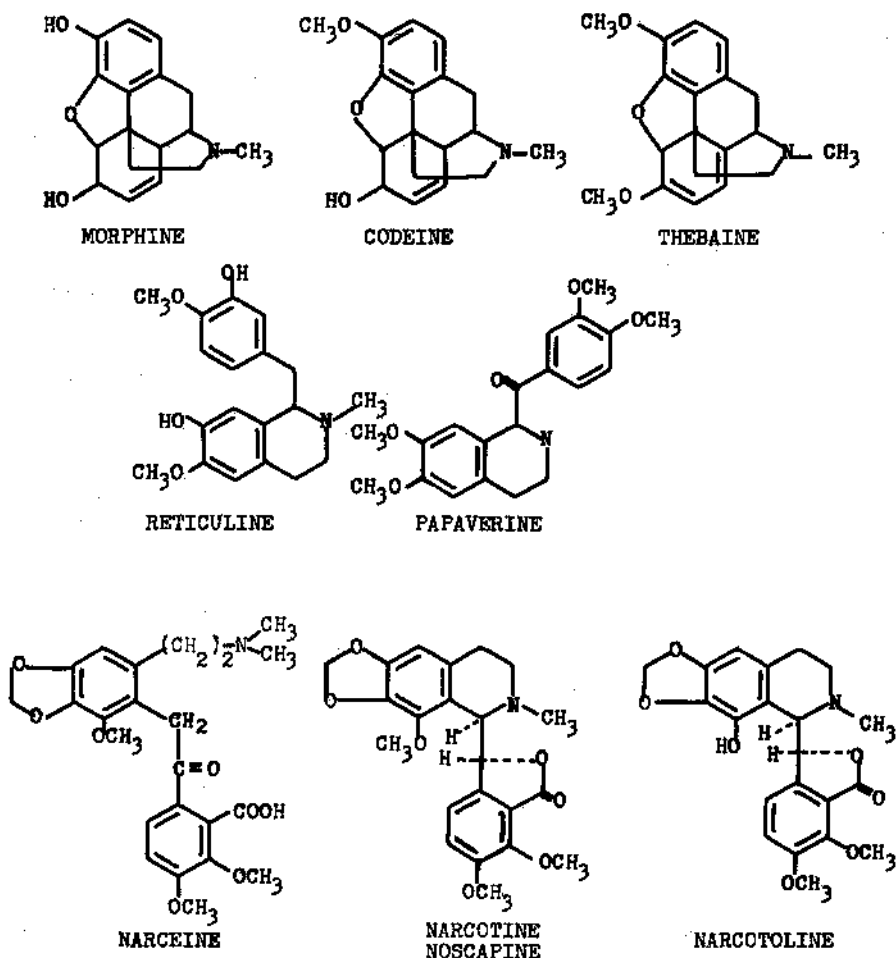


FIGURE 3. Chemical structure of alkaloids present in seedlings of poppy (*Papaver somniferum* L.).

3rd day group.

Seventh, 8th and 9th day seedlings contained the same compounds as reported present in the previous extracts, but in still larger amounts. This was indicated by spots more intense as well as of larger total area. The extract from 8th day seedlings had in addition to the other compounds another spot below morphine and below codeine. These spots were not seen on plates from the other extracts and their identity was not known. In another context "morphine like" and "codeine like" substances are reported (Fairbairn and El-Masry, 1968). These compounds might be of this type.

It is interesting to note that the alkaloids present in the early development of the seedling of poppy are the same as those present in the developing capsule—the period when production is at its peak. This is true not only for the major alkaloids, but also for minor ones that might be expected to vary widely during ontogeny of the plant. This might imply that the effect of environment on alkaloid production is not as great as is encountered in some of the other plants studied, and may even indicate that environmental variables such as fertilizer treatments that often merit major consideration in influencing alkaloid synthesis is of less importance where poppy alkaloids are concerned.

It is of interest further to note that Fairbairn and Kapoor (1960) who worked with the anatomy of poppy seedling, growing plant and developing capsule, found latex vessels at the stage where cotyledons expanded and became green. They did not find any in seedlings young-

er than this stage. They indicated the appearance of these vessels as at 8 days, although this stage of cotyledonary development was reached by the 4th day. This would imply that the synthesis of alkaloids is intimately associated with the development of the latex vessels. In contrast to certain other alkaloid producing plants where synthesis is associated with actively dividing root apical cells, it would appear that developing laticifers are the site of synthesis of the poppy alkaloids.

It was not possible to measure accurately the amounts of alkaloids present in the extracts that were chromatographed. Massicot (1961) reported measurement of 16 μ g. of thebaine from 100 7th day seedlings. He applied the separate compounds which result in the Dragendorff-alkaloid complex to filter paper sheets separately, allowing the complex to form on the paper at the point where alkaloids were located after chromatography, thus making a far more sensitive measure than usual. Several careful attempts to repeat his method on paper and apply it to thin-layer determination were unsuccessful. Though concentrations of morphine and codeine, and the high concentration of thebaine reported by Massicot appear to be in agreement with our results, it appears strange that he did not report also the large amounts of papaverine and narcotine which were also found by the thin-layer method of separation.

Attempts were also made in this study to feed C^{14} sucrose and tyrosine to seedlings during a period of growth followed by killing, extrac-

tion and separation by TLC, and the determination of radioactivity in various constituents. Though large amounts of radioactivity from sucrose and tyrosine were fixed into tissue constituents, the amount incorporated into alkaloids was so small that this means of measurement was not considered satisfactory.

Of particular interest to workers in the area of public health is the observation of Fairbairn and El-Masry (1968) that a form of "bound" morphine is present in the seed of poppy. Though large amounts of poppy seeds are commonly used in cooked and baked foods, few references have been made to untoward pharmacological effects, and these are presumably due to latex of the fruit wall contaminating seeds in preparation. Where clean seeds were ground and exposed to vigorous acid hydrolysis or acid-pepsin digestion for several hours, codeine and several morphine-like substances were shown to be present in the seeds. These compounds are presumably bound to a protein or other large molecule and

are probably not hydrolyzed by stomach acid in a short time. Widespread interest will attend further reports of this seed-alkaloid relationship.

ACKNOWLEDGMENTS

This work was done during a Sabbatical Leave from the University of Illinois of F. A. Crane in the laboratory of J. W. Fairbairn at the School of Pharmacy, University of London, 1967.

LITERATURE CITED

- FAIRBAIRN, J. W. and S. EL-MASRY. 1968. The Alkaloids of *Papaver somniferum* L. VI. "Bound" Morphine and Seed Development. *Phytochem.* 7:181-187.
- FAIRBAIRN, J. W. and L. D. KAPOOR. 1960. The Laticiferous Vessels of *Papaver somniferum* L. *Planta Medica* 8:49-61.
- GENEST, K. and C. G. FARMILLO. 1962. Simultaneous determination of morphine, codeine and porphyroxine in opium by infrared and visible spectrometry. *Anal. Chem.* 34:1464-1468.
- MARY, N. Y. and E. BROCHMANN-HANSEN. 1963. Quantitative Determination of the Principal Alkaloids of Opium by Thin-Layer Chromatography. *Lloydia* 26:223-228.
- MASSICOT, J. 1961. Biosynthesis of the Alkaloids in the Seedlings of *Papaver somniferum*. *Ann. Pharmac. Franc.* XIX:44-52.

Manuscript received, June 13, 1969