

EXTRACTION AND IDENTIFICATION OF CLAVINE AND LYSERGIC ACID ALKALOIDS FROM MORNING GLORIES¹

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Seeds from three varieties of *Ipomoea* grown in Ohio were examined for clavine and lysergic acid alkaloids. Three systems of extraction of ergoline alkaloids were compared with the ammonium hydroxide-methanol-chloroform method proving superior to the others. Four chromatographic systems were compared for ability to resolve and identify ergoline alkaloids. Of the four systems, a thin-layer system with ethyl acetate-ethanol-dimethyl-formamide gave the best resolution of ergoline alkaloids. *Ipomoea violacea* L. var. Pearly Gates had significantly more total alkaloids and more individual ergoline alkaloids.

For many years, scientists have known of a series of hallucinogenic alkaloids in such unrelated plants as cactus, mushrooms and morning glories. There is evidence that some groups of Indians have used various of these plants for ceremonial purposes for centuries (MacDougall, 1960; Schultes, 1941), although there is no record of use by Indians of Ohio. MacDougall believes that tribes of Indians used the morning glory in religious ceremonies and as a type of narcotic or analgesic. In the early 1570's, the King of Spain's personal physician, Hernandez, wrote that the Aztecs used morning glory seeds, called Ololiuqi, to commune with their gods (Hofmann, 1968).

In the 1930's, the Sandoz Pharmaceutical Company of Basle, Switzerland, extracted a number of pharmacologically active substances from the ergot fungus, *Claviceps purpurea*. These substances were lysergic acid derivatives and clavine

alkaloids. These substances are sometimes called ergoline compounds (Der Marderosian, 1967). In 1938, Albert Hofmann of the Sandoz Company, synthesized LSD from ergoline compounds, and in 1960 he discovered lysergic acid and clavine alkaloids in morning glory seeds of *Ipomoea violacea* Sp. (Hofmann, 1968).

A number of investigators, since Hofmann, have identified ergoline compounds in *Ipomoea* Sp. (Genest, 1966; Niwaguchi and Inoue, 1969; Taber et al., 1963). The literature records a wide range of qualitative and quantitative data on ergoline compounds in morning glories. These data are confused by the variety of extraction and identification techniques and differences in preparation techniques. In trial runs in our laboratory, it was discovered that quantitative results varied from seed batch to seed batch, and that time and technique of extraction and chromatographic separation was very important.

METHODS

Seeds were collected from three types of morning glory *Ipomoea violacea* L. var. heavenly Blue, *Ipomoea violacea* L. var. Pearly Gates, and *Ipomoea violacea* L. var. Blue Star (based on the classification of Der Marderosian 1967) grown from seeds purchased from the George W. Park Seed Co., Greenwood, North Carolina.

Three methods of extraction of ergoline alkaloids were compared using 5-g portions of each of the three varieties of seed, ground with clean quartz sand in a mortar. Total ergoline alkaloid content was estimated by colorimetric procedures developed by Genest (1966) and Vining and Taber (1959). The methods for extraction were:

1. Ground seeds were washed with 50 ml of a 1:1 mixture of 10% sodium bicarbonate and ethyl acetate. The solvent fraction was removed from the seeds and dried. The residue was treated with 50 ml of a 1:1 mixture of diethyl ether and 1% tartaric acid. The alkaloids were present in the lower layer (Taber et al., 1963).

2. The ground seeds were covered with a 10% ammonium hydroxide in diethyl ether mixture, the solvent was dried and the residue was

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dissolved in a 1:1 mixture of diethyl ether and 1% tartaric acid (Taber et al., 1963).

3. The ground seeds were treated with 50 ml of a 10:90:900 mixture of ammonium hydroxide, methanol and chloroform. The slurry was shaken for 5 minutes, and 50 ml of chloroform and 2 ml of water was added. The slurry was filtered through glass wool and the solvent collected (Alexander and Baner, 1961).

Solutions from each of the three above procedures were treated with chloroform after neutralizing each with excess sodium bicarbonate. The chloroform was removed under vacuum and the residue redissolved in 0.5 ml ethanol.

Isolation and identification of the extracted ergoline alkaloids was done by four chromatographic methods. Exactly 50 microliters of each solution was placed as a single spot on paper strips or thin-layer plates. Procedures for identification and separation of the ergoline alkaloids were:

1. Whatman No. 3 MM paper was impregnated with a solution of 4% benzoic acid in a 3:1 mixture of methanol and formamide. After the paper was dry an aliquot of ergoline-containing solvent was spotted on the paper and the chromatogram developed in a mixture of benzene-pyridine (6:1) for 3.5 hours (Agurell and Ramstad, 1962).

2. Whatman No. 3 MM paper was impregnated with the solution of 4% benzoic acid in 3:1 methanol-formamide. The alkaloid-containing solution was spotted on the paper and developed in a mixture of chloroform-pyridine (6:1) for two hours (Agurell and Ramstad, 1962).

3. Thin layer plates were covered with a 0.25 mm layer of silica gel G and activated. The alkaloid solution was spotted at the baseline and they were developed in a 13:1:1 mixture

of ethyl acetate-ethanol-dimethylformamide (Agurell and Ramstad, 1962).

4. Thin layer plates were covered with a 0.25 mm layer of silica gel G and activated. The alkaloid mixture was spotted on the plates and the plates were developed in a 5:5:1 mixture of acetone-ethyl acetate-dimethylformamide (Niwauchi and Inoue, 1969).

Ergoline standards (Aldrich Chemical Co.) were added to each chromatogram to compare with the unknown spots. The location of the ergoline alkaloids was determined by noting the fluorescent zones that appeared under ultraviolet light and also by spraying the completed chromatogram with van Urk's reagent (1 g 4-dimethylaminobenzaldehyde dissolved in a mixture of 50 ml 25% hydrochloric acid and 50 ml ethanol) (Waldi, 1962).

RESULTS AND DISCUSSION

Of the three varieties of *Ipomoea* investigated, *Ipomoea violacea* L. var. Pearly Gates had a significantly higher yield of total alkaloids in all three systems of extraction (table 1). The Pearly Gates variety yielded a mean value of 0.060% of fresh weight as compared to mean values of 0.034 and 0.027% for the Heavenly Blue and Blue Star varieties, respectively. The extraction system using ammonium hydroxide, methanol and chloroform developed by Alexander and Baner (1961) gave the highest mean yield of ergoline alkaloids by fresh weight from the three varieties of morning glory, compared to the other two systems. The

TABLE 1
Alkaloid content of *Ipomoea* seeds.¹

Type of <i>Ipomoea</i>	% Fresh weight in 3 extraction systems ²			
	1	2	3	Mean
Heavenly Blue	0.028	0.036	0.038	0.034
Pearly Gates	0.040	0.068	0.072	0.060
Blue Star	0.022	0.026	0.032	0.027
Mean:	0.030	0.043	0.047	

¹The values are expressed as ergometrine equivalents using the van Urk reagent (Vining and Taber, 1959).

²Extraction method: 1. 10% sodium bicarbonate in ethyl acetate; the extract was dried and the residue taken up in a mixture of ether and 1% tartaric acid.

Extraction method: 2. 10% ammonium hydroxide and ether. Extract was dried and residue taken up in ether and 1% tartaric acid.

Extraction method: 3. 50 ml of a mixture of 10:90:900 ammonium hydroxide, methanol and chloroform to which a 50 ml aliquot of chloroform and 2 ml of water was added.

mean per cent fresh weight of alkaloids extracted by the ammonium hydroxide, methanol and chloroform mixture was 0.045, while the ammonium hydroxide in diethyl ether solution yielded 0.43, and the sodium bicarbonate, ethyl acetate solution yielded only 0.030 per cent of fresh weight for the ergoline alkaloids.

The Pearly Gates variety had the most ergoline alkaloids in all three extraction systems (table 1) with a per cent fresh weight of ergoline compounds of 0.060 compared to 0.034 and 0.027 for the Heavenly Blue and the Blue Star varieties, respectively. All six of the alkaloids listed in Table 2 were found in Pearly Gates with the paper chromatographic system in which the paper was impregnated with 4% benzoic acid in a 3:1 mixture of methanol-formamide and developed in chloroform-pyridine, and with the thin-layer chromatographic system that used a 13:1:1 mixture of ethyl acetate-

ethanol-dimethylformamide. In Pearly Gates, ergometrine and agroclavine were not detectable in the paper chromatographic system using benzene-pyridine as the developing mixture, and penniclavine was not found using the thin-layer system using a 5:5:1 mixture of acetone, ethyl acetate and dimethylformamide.

In the Heavenly Blue seeds, no chanoclavine or penniclavine was detected using any of the four chromatographic methods. No agroclavine, penniclavine, or chanoclavine were found in Blue Star seeds.

The thin-layer systems using the mixture of ethyl acetate, ethanol and dimethylformamide gave the greatest clarity of individual spots and had the further advantage of taking less than two hours, whereas the paper chromatographic systems took nearly three hours.

Ipomoea violacea L. var. Pearly Gates was found to give the highest per cent

TABLE 2
R_F values of extracts from three varieties of *Ipomoea*.

	Chromatographic system ¹				
	1	2	3	4	
<i>Ipomoea violacea</i> L. Heavenly Blue	—	—	—	—	chanoclavine
	0.47	0.28	0.15	0.23	elymoclavine
	—	—	—	—	penniclavine
	0.67	0.18	0.23	0.28	ergometrine
	0.76	0.70	0.40	0.52	agroclavine
<i>Ipomoea violacea</i> L. var. Pearly Gates	0.62	0.75	0.47	0.26	lysergic acid amide
	0.21	0.12	0.10	0.42	chanoclavine
	0.48	0.28	0.18	0.24	elymoclavine
	0.44	0.22	0.46	—	penniclavine
	—	0.18	0.24	0.32	ergometrine
<i>Ipomoea violacea</i> L. var. Blue Star	—	0.70	0.42	0.54	agroclavine
	0.62	0.78	0.48	0.30	lysergic acid amide
	—	—	—	—	chanoclavine
	0.46	0.28	0.21	0.23	elymoclavine
	—	—	—	—	penniclavine
	0.70	0.20	0.24	0.35	ergometrine
	—	—	—	—	agroclavine
	0.60	0.77	0.46	0.31	lysergic acid amide

¹Procedure for Identification and Separation 1. Whatman No. 3 paper impregnated with 4% benzoic acid in a mixture of methanol-formamide (3:1). Developed in a 6:1 mixture of benzene-pyridine for 3.5 hours. 2. Whatman No. 3 paper impregnated as system 1 but the paper was developed in a 6:1 mixture of chloroform-pyridine for two hours. 3. Thin-layer plates coated with silica gel G were developed in a mixture of ethyl acetate, ethanol, dimethylformamide (13:1:1). 4. Thin-layer plates coated with silica gel G were developed in a mixture of acetone, ethyl acetate, dimethylformamide (5:5:1).

(0.60) yield of clavine and lysergic acid alkaloids of the three varieties of morning glory seeds tested. Variety Heavenly Blue gave the second highest (0.034) and Blue Star gave the least (0.027 (Table 1). Taber et al. (1963), using similar techniques of quantification, found per cent fresh weight values of 0.057, 0.042 and 0.024 respectively for seeds of *Ipomoea rubro-caerulea* L., *Ipomoea alba* L., and *Ipomoea violacea* L. The seeds used by Taber et al. were from England. Der Marderosian (1965) and Gunn (1972) believe that *I. rubro-caerulea* L. and *I. alba* L. are varieties of *Ipomoea violacea* L. Alba is now considered to be the Pearly Gates variety.

Very little work has been done on the environmental influences of soil, temperature, rainfall and other factors on the development of alkaloids in the morning glories. It is interesting to note that there are variations in ergoline content from seed batch to seed batch, requiring multiple sampling for proper analysis.

The extraction systems using 10% ammonium hydroxide in diethyl ether and a 10: 90: 900 mixture of ammonium hydroxide, methanol and chloroform gave higher yields than treatment with sodium bicarbonate and ethyl acetate. Taber et al. (1963) found somewhat different results with a mixture of ammonium hydroxide, methanol and chloroform (10:90:900).

Chanoclavine was not found in *Ipomoea violacea* L. var. Heavenly Blue. Genest (1966) found chanoclavine in immature seeds and trace amounts in mature seeds and suggested that through a series of steps it could be converted to lysergic acid amide. Agurell and Ramstad (1962) showed that there is a definite conversion of clavine alkaloids to lysergic acid-related compounds. The seeds used in this experiment were mature, and it is possible that chanoclavine may have been present in the immature seeds. In our study, there were substantial spots of agroclavine on thin-layer plates treated with extracts from both Heavenly Blue and Pearly Gates. Genest (1966) could

find no agroclavine in *Ipomoea violacea* L. var. Heavenly Blue. The reason for this difference is not clear.

Thin-layer chromatography is a more effective and rapid method for separating and identifying alkaloids than the paper chromatographic methods. Environmental and genetic factors may have been involved in some of the variations between seed batches and should be further investigated. Other factors such as maturity and age of seeds should also be considered.

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