Identification of Seeds of Ipomea Purpurea (Morning Glory Family reported to have Psychotomimetic Properties) by Paper Chromatography

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This paper describes a paper chromatographic method of identification of morning glory (Ipomoea purpurea) seeds used by individuals seeking hallucinatory experiences. The procedure is applicable to a single seed, seed fragments and products of "tea".

A 50 per cent aqueous alcoholic extract is chromatographed in a butanol : acetic acid : water system and the pattern visualized by ultraviolet fluorescence, p-dimethylaminobenzaldehyde and ninhydrin spray reagents. The chromatographic patterns of various morning glory seeds and other seeds of criminalistic interest are shown. Milligram quantities of Ipomoea purpurea (two varieties) are readily differentiated from all other seeds examined.

(Note : Authors request all correspondence be directed to Paul M. Dougherty).

The reports that the seeds of *Ipomoea purpurea* contain psychotomimetic substances have prompted their use by individuals seeking a "psychedelic" experience. The presence of seeds in submitted evidence makes it necessary for the criminalistics laboratory to provide a rapid and sensitive method for their identification both as entire seeds and under altered conditions, i.e., fragmented, leached after boiling in water as in preparation of a "tea", fermented or as a residue in a container.

The conventional methods of identification by morphological examination of the specimen or by actual planting of the seed are not possible unless the seed is nearly intact ; in addition, the time element precludes planting in most forensic situations. Identification might be made by a microscopic study of the internal structure of the seed by a taxonomist. Identification by recognition of alkaloids present using Van Urk's reagent (Taber and Heacock, 1962), or by fluorometric characterization of extracts of the seeds (Vining and Taber, 1959) are nonspecific and have not been applied to altered material.

The active principles contained in the Mexican type seeds of *Rivea corymbosa* and *Ipomoea violacea* are reported to be ergot type compounds. These include d-lysergic acid amide, d-iso-lysergic acid amide, and chanoclavine (Hofmann and Tscherter, 1960). Elymoclavine, lysergol and ergonovine (ergometrine) were also reported present by Hofmann et al.

The use of chromatography by these investigators to isolate and identify these alkaloidal compounds in Morning Glory seeds initiated our efforts to identify the seeds themselves through their constituent compounds by these means.

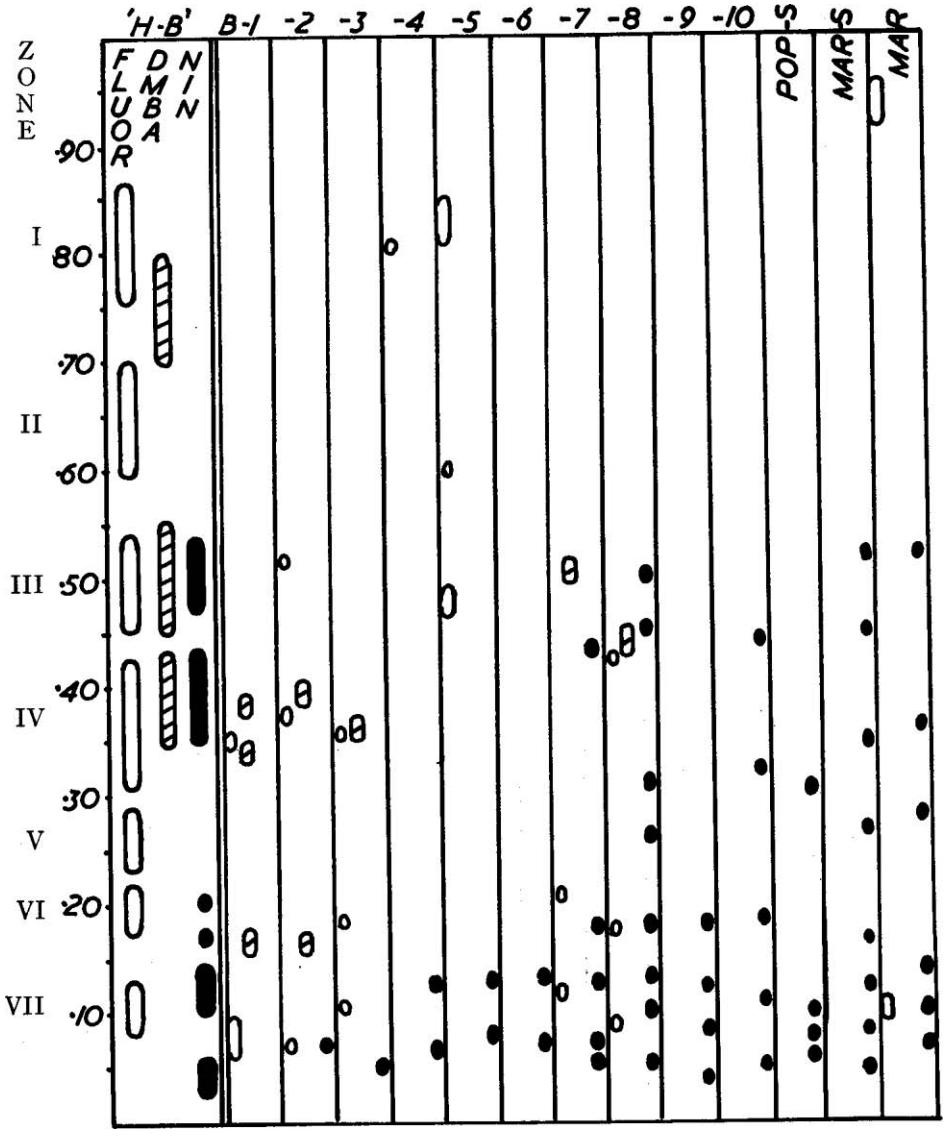

Figure 1 Composite chromatogram of *Ipomoea purpurea* (var. Heavenly Blue) and miscellaneous other seeds. The seeds are identified as follows : B1 to B10 are seeds separated from a sample of commercial wild bird food, POPS-S is commercial poppy seed (Schilling), MAR-S is Marijuana seed (*Cannabis sativa*) and MAR is dried Marijuana plant. On the left of each column is shown the ultraviolet fluorescence response. In the centre of each column is the response to DMBA reagent. On the right of each column is the ninhydrin response. Rf values are shown on the left.

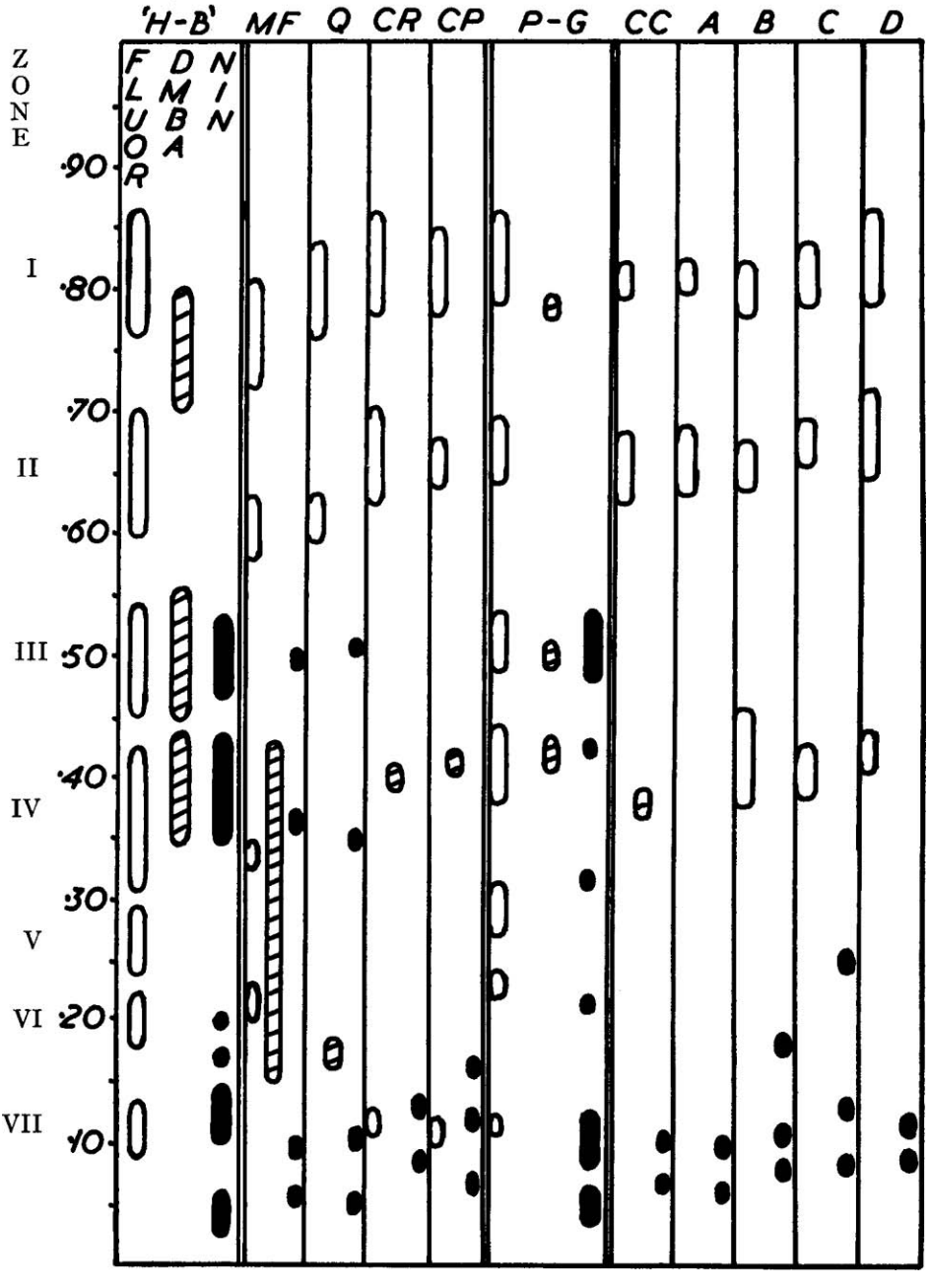

Figure 2 Composite chromatogram of Heavenly Blue and other Morning Glory Seeds. Seeds are identified at top as follows: HB, *Ipomoea purpurea*, var. Heavenly Blue; MF, *Ipomoea*, "Moon flower"; Q, *Ipomoea*, "Quamoclit"; CR, *Ipomoea*, "Crimson Rambler"; CP, *Ipomoea*, "Candy Pink"; PG, *Ipomoea purpurea*, var. "Pearly Gates"; CC—*Ipomoea*, "Cardinal Climber". A, B, C, D, Seeds separated from package of commercial Morning Glory seeds. On left of each column is shown fluorescence response in ultraviolet light. In center of each column is response to DMBA reagent. On right of each column in Nin response. Rf values on extreme left of figure.

A 50% alcohol-water extract of *Ipomoea purpurea* var. Heavenly Blue (HB) was applied to produce a paper chromatogram using a n-butanol, acetic acid, water developing solvent system. Fluorescent zones were observed and the chromatogram was then sprayed with p-dimethylaminobenzaldehyde reagent (DMBA). A second chromatogram was treated with ninhydrin (Nin) reagent. Characteristic, reproduceable patterns resulted.

To determine specificity, ten different kinds of seed were mechanically separated from a commercial "Wild Birdseed" mix, and together with commercial poppy seed, marijuana seed, marijuana and HB were extracted and compared in the same manner. Results indicated all of the samples could be readily distinguished from each other and from HB (see figure 1). Six other seeds of the genus *Ipomoea* were obtained from a commercial source, and together with four kinds of seeds mechanically separated from a packet of "Morning Glory" mix of another commercial source, were compared with HB, in the same manner (see figure 2). Only one of these seeds produced a result the same as that of HB. This was expected as this seed, PG, is a variety of *Ipomoea purpurea*, and has been reported as containing indole alkaloids and producing psychotogenic effects. (Beyerman, et al., 1963). Identification was found to be possible using milligram amounts of the HB seeds, even under various conditions of fragmentation, desiccation, heat treatment, and leaching as described below.

Experimental

Extraction techniques Procedure A

1.0 gram of the seed was crushed with thin nosed pliers and placed in a mortar. 8 ml of alcohol-water (1:1) was added, ground for 2-3 minutes, and allowed to settle for 15 minutes. The liquid mixture was added to a centrifuge tube. The pulp remaining in the mortar was ground with two additional 3 ml portions of solvent allowed to settle, and added to the tube, making 12 ml of slurry. The mixture was centrifuged until clear. The oil layer was discarded and the supernatant liquid was filtered. Two washes, each with 12 ml of solvent, were made of the residue in the tube, resuspending the solids, and centrifuging. The oil was discarded, the supernatant layer removed, filtered, and added to the intial extract . The entire extract was then evaporated to near dryness on a steam bath, allowed to air dry, producing a yellowish waxy residue.

Extraction Technique—Procedure B (short method)

1.0 gram of seed was crushed and ground very fine. 6 ml of 1:1 alcohol-water solvent was added and the whole ground for 2-3 minutes, let stand 5 minutes, and the light slurry added to a centrifuge tube. This process was repeated, the combined extracts in the tube shaken for 2-3 minutes, and centrifuged until clear. The liquid was filtered, and evaporated as in Procedure A.

Application

The residue is taken up in 1 ml of 1:1 alcohol-water, and approximately five microliters (equivalent to 5 micrograms of original seed) applied to paper. In some trials, 10 or 15 microliters were used.

Paper Chromatography

(a) chamber equilibration :

9.5 ml n-butyl alcohol, 5.2 ml glacial acetic acid, water to make 100 ml. (may form two phases).

(b) development :

23 ml water, 6.9 ml glacial acetic acid, .75 ml n-butyl acetate, n-butyl alcohol to make 110 ml (monophasic).

(c) conditions :

Whatman Number One paper is used. Extracts are applied, air dried and the paper equilibrated four hours. The developing solvent is allowed to run about 180 cm ; time, about 14 hours. A mixture of approximately 10 micrograms each of morphine sulfate, codeine sulfate, and heroin hydrochloride was placed on each chromatogram for reference purposes and sprayed separately with the iodoplatinate reagent.

(d) chromatogenic reagents :

1. p-Dimethylaminobenzaldehyde (DMBA) ; 1.0 g in 8 ml 37% HCl, diluted to 50 ml with water (Biochem. J. 1952) LSD and ergot alkaloids produce blue-purple spots after a short time without heat.
2. Ninhydrin (Nin) 0.5% solution in acetone. The a-amino acids give gray-purple spots on standing overnight.
3. Potassium iodoplatinate. 10 ml of platinic chloride dissolved in 250 ml of 4% potassium iodide, diluted to 500 ml with water. (Munier and Machboeuf, 1949). Reference standards produce blue-purple spots on pink background. No reaction was obtained with this reagent with extracts of HB.
4. Ferric chloride-Ferricyanide. 0.3% solutions of ferric chloride and potassium ferricyanide, each diluted tenfold, and mixed 1:1. After spraying the chromatogram is further sprayed with dilute hydrochloric acid and washed with water. (Barton et al. 1952). Phenols, tannins, and other reducing substances produce blue spots. Fluorescent zones of HB and PG gave blue spots with this reagent.
5. The following chromatogenic reagents were tried but found to be of no value for our purposes : Ninhydrin-acetic acid, xanthydrol, diazotized sulfanilic acid, diazotized p-nitroaniline, formaldehyde-hydrochloric acid, and silver nitrate in strong alkali.

Discussion

The 50% alcohol water solution was selected because of its general solvent characteristics, and also because it dissolves one class of proteins (prolamins) that occurs in many common seeds, wheat, rye, barley, etc.

An aqueous ammonia-chloroform procedure was tried in the early tests, but proved much inferior to the alcohol-water system, and was abandoned.

Extraction procedure A was used in tests of "Wild Bird" seed, (B-1 through B-10 inclusive), poppy seed, (Pop-S), marijuana seed, (Mar-S) and marijuana (Mar) (see figure 1). Extraction procedure B gives identical responses with HB as procedure A ; hence, extraction procedure B was used in all other tests (see figure 2).

Tests of the oils extracted from the seeds revealed that it was without significant effect on the chromatogram ; therefore the oil was disregarded in procedure B.

An acetic acid-n-butyl acohol paper chromatographic system formulated in this laboratory was used in this work because it has a wide range of applicability ; i.e., it is an excellent general purpose system.

Results

(1) The "identification pattern" for HB is shown on figures 1 and 2, and consists of :

(a) seven blue white fluorescent areas :

(b) three DMBA blue-purple spots

(c) three Nin gray purple spots present at zones III, IV, and VII, plus other spots, generally of low Rf values.

This pattern of fluorescent and color spots is the result of over 70 determinations of the Rf values of HB. The average Rf values of each spot and range is indicated in the figures. Seeds from two lots of HB from same commercial source were identical.

The DMBA and Nin values indicated for the other seeds are in most cases single runs. The Rf values and ranges for the fluorescence are for a minimum of three runs, with the exception of PG where six trials are indicated in the figures.

It will be noted that the patterns for all the seeds with the exception of "Pearly Gates" (PG) are entirely different from that of HB. The pattern of PG appears to be nearly identical with that of HB.

2. Seeds of HB were coarsely crushed and separated into portions consisting of dark coated outer layer and white inner pulp of seed. Chromatograms of extracts of each portion showed the extractable material which is the basis for identification is all in the pulp.

3. An extract of HB after two and then three months standing intermittently opened and closed to the air produced approximately the same pattern as when chromatographed at the time of its preparation.

4. "Ageing" of the HB was simulated by grinding the seeds, permitting to stand exposed to the air for 10 days, one sample at room temperature and another at 100C. Chromatographic responses from these trials indicated a slight lowering of the Rf values from the average for the fluorescent spots in zones I and II, and a lower than average Rf value for the top DMBA spot. There was no reduction in the fluorescence or color intensity. The Nin spots were normal.

5. Processing of the HB (both whole seed and crushed) with hot and boiling water as in the preparation of a "tea", appeared to extract the same components from these seeds as with the 50% alcohol-water solvent. This is indicated from the fluorescence and DMBA color responses. The Nin responses at zones III and IV failed to appear although the other Nin responses were present in the "tea" in those trials, as well as from the leached seeds. A second extract of the leached seeds and resulting tea continued to give a weak but nevertheless detectable response, similar to the patterns from the first extraction.

6. A 2.0g sample of HB was crushed and warmed in 100 ml water of the steam bath for 4-5 hours, then let stand for 10 days with intermittent warming. Fermentation and mold formation occurred. The semi-liquid mass resulting as well as the heavy decomposed solid material, when extracted with the solvent produced standard HB patterns.

7. Two single HB seeds were each crushed and then extracted separately on the spot-plate with the 50% alcohol-water solvent. In each case, as much as possible of the extract was transferred to the chromatographic paper for developing. The pattern response for each seed was adequate for identification.

8. Employing the usual extraction procedure and dissolving the product in 1 ml of alcohol-water (1:1), a 5 microgram application was made on the chromatographic paper. The volume of the extract was then successively increased to 2.0, 5.0 and 10 ml with the solvent, 5 microliters of the extract applied after each dilution. A standard response was obtained on the 2.0 ml volume except there was no Nin color response. For the 5.0 ml volume, the response was faint but discernable. The 10 ml dilution produced no DMBA or Nin color response but did give a faint standard fluorescence pattern.

9. The amount of material extracted from two separate 1 gram samples of HB seeds was 6.4% and 7.1%; average 6.8%. It is estimated on this basis that 2 mg of seed is sufficient for identification.

None of the extractable material was chemically identified.

It is believed as a result of this limited work that a system of chromatographic patterns, if sufficiently unique, as is that of HB, provides a simple procedure for the identification of seed, seed fragments and vegetable material impractical or impossible by other means.

Acknowledgements

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Note added in proof:

Attention of readers interested in this subject are directed to the following article:

GENEST, K., 1965, *J. Chromatog.*, **19**, 531-539.