Morning Glory Tissue Cultures: Growth and Examination for Indole Alkaloids

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The seeds and aerial portions of three *Ipomoea violacea* varieties contained significant amounts of indole alkaloids. The roots, callus tissue, and callus medium of these three varieties; the callus tissue and callus medium of *Rivea corymbosa;* and the seeds of three Japanese morning glory varieties contained traces of indole alkaloids.

IT HAS BEEN conclusively established that some *Convolvulaceae* plants contain ergot-type alkaloids. Among them are the seeds of *Argyreia nervosa* (baby Hawaiian wood rose) (1) and *Ipomoea argyrophylla* Vatke (2), *I. coccinea* L. (3), *I. muelleri* Benth. (4), *I. rubro-caerulea* Hook (5) [*I. violacea* (6)]; the seeds (7, 8) and plants (9) of *I. violacea* L. (morning glory, Badoh Negro, *I. tricolor);* and the seeds (7) and plants (10) of *Rivea corymbosa* (L.) Hall. f. (Ololiuqui, wild morning glory).[1]

Certain varieties of *I. violacea* are reported to induce a physiological response similar to lysergic acid diethylamide (11), although a recent study indicates sedation is the principal physiological effect of *I. violacea* and *R. corymbosa* seeds (12). Crude seed extracts of either heavenly blue or pearly gates varieties of *I. violacea* will cause isolated rat uterus muscle to contract (11). Sheep fed dried *I. muelleri* plants for 3 to 6.5 weeks lost weight, became incoordinated, and acquired a rapid panting respiration (4).

The principal objective of this study was to examine three varieties of *I. violacea* and *R. corymbosa* seed callus tissue cultures for indole alkaloids by thin-layer chromatography and spectrophotometry. In addition, the seeds and plants of these three varieties, and the seeds of three varities of Japanese morning glory were examined for indole alkaloids.

MATERIALS AND METHODS

The source of seeds for this study were: *Ipomoea violacea* L. cv. flying saucer (FS), cv. heavenly blue (HB), and cv. pearly gates (PG) (Park Seed Co., Greenwood, S. C.); *Ipomoea nil* (L.) Roth [*Pharbitis nil* Chois, Japanese morning glory (13)] cv. matzukaze, yuki, and chiyo no okina (H. Saier Seedsman, Dimondale, Mich.); and *Rivea corymbosa* (L.) Hall. f. (Prairie Regional Laboratory, Saskatoon, Canada, who purchased them from Atkins Garden and Research Laboratory, Cienfuegos, Cuba). Plants studied were grown from seeds in the Drug Plant Greenhouse, University of Nebraska.

Tissue Cultures.—*Ipomoea* seeds were sterilized in a 5.25% sodium hypochlorite solution (Purex Corp., Ltd., South Gate, Calif.) diluted 1:1 with sterile distilled water for 15 min. in vacuum, and *Rivea* seeds in a 1:3 dilution for 10 min. in vacuum. An unusually strong seed sterilization treatment was required for *Ipomoea* seeds to avoid mold contamination. As the seeds normally germinated within 2 days, they were directly transferred, after thorough rinsing with sterile distilled water, to 1-oz., square glass vials containing 18 ml. of Murashige's and Skoog's tobacco medium (14) with 1.0 p.p.m. 2,4-dichlorophenoxyacetic acid (2,4-D) and 1.0% agar.

Extraction Procedure.—Plant, seed, callus tissue and agar medium were extracted for indole alkaloids by a modification of the procedures published by Taber *et al.* (9) and by Abe and Yamatodani (15).

The aerial and root portions were ground in a Wiley mill to a 40-mesh powder; whereas, the seed and dried callus tissue were ground with a mortar and pestle. The plant samples (seed, 2.0 Gm.; aerial, 3.0 Gm.; root, 6.0 Gm.; callus tissue, amount indicated in Table I) were wetted with 10% ammonium hydroxide and allowed to macerate overnight. The ground seeds were first wetted with ethyl ether to facilitate more thorough wetting of the seeds by alkali. The samples were then extracted with ethyl ether in a Soxhlet apparatus for 24 hr.

The agar media were diluted approximately 3:1 with water, made alkaline with 10% ammonium hydroxide, and extracted for 2 to 3 days with ethyl ether in a liquid–liquid extractor.

Each ethereal fraction was then evaporated to dryness in vacuum and the residue dissolved in 10 ml. of 0.2 *N* sulfuric acid. The acidic solution was washed with 10-ml. portions of ethyl ether to remove residues and color. The acidic solution was then neutralized with an excess of sodium bicarbonate and extracted with three 15-ml. portions of chloroform (fraction A). The aqueous solution was then adjusted to pH 6.8 with 0.2 *N* sulfuric acid and again extracted with three 15-ml. portions of chloroform (fraction B). The remaining aqueous layer was filtered and designated fraction C. Fractions A, B, and C were divided into equal aliquots—A_1 and A_2; B_1 and B_2; and C_1 and C_2. Aliquots A_1, B_1,

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[1] Some of these species contain the lysergic acid-type alkaloids ergosine (2); ergometrine (ergonovine), ergine, and isoergine (7); ergometrinine, ergosinine, ergotamine, and lysergic acid (9) and/or the clavine-type alkaloids agroclavine (2); chanoclavine, elymoclavine, and lysergol (7); and penniclavine (9).

TABLE I.—ALKALOID CONTENT OF MORNING GLORY SEEDS, PLANTS, AND TISSUE CULTURES[a]

Plant Variety	Plant Age, Months	Seeds Fraction[b] A	Seeds Fraction[b] B	Aerial Fraction[b] A	Aerial Fraction[b] B	Roots Fraction[b] A	Roots Fraction[b] B	Callus Age, Months[c]	Callus Tissue Dry Wt., Gm.	Callus Fraction[b] A	Callus Fraction[b] B	Agar Medium Agar Wet Wt., Gm.	Agar Medium Fraction[b] A	Agar Medium Fraction[b] B
Heavenly blue	5	0.486	0.007	46.0	0.9	13.3	1.3	15	7.1	0.0	0.0	1259	Trace	0.0
Heavenly blue	4	0.296	0.006	22.6	2.2	...	...	...	...	...	...	...	...	...
Flying saucer	5	0.240	0.039	Trace	0.9	1.3	2.0	15	6.8	0.6	0.6	987	0.0	8.0
Flying saucer	4	...	...	2.6	0.9	...	...	...	...	...	...	...	...	...
Pearly gates	5	0.053	0.003	132.0	2.2	4.0	1.3	15	9.2	0.2	0.4	804	4.0	4.0
Pearly gates	4	...	...	30.0	1.7	...	...	...	...	...	...	...	...	...
Rivea	...	...	...	...	...	...	...	6	18.7	1.0	2.8	490	Trace	0.0
Rivea	...	...	...	...	...	...	...	15	8.2	2.2	1.0	425	0.0	Trace
Matzukaze	...	0.005	0.002	...	...	...	...	...	...	...	...	...	...	...
Yuki	...	0.006	0.005	...	...	...	...	...	...	...	...	...	...	...
Chiyo no okina	...	0.007	0.004	...	...	...	...	...	...	...	...	...	...	...

[a] Alkaloid content for seeds expressed as mg./Gm. of seed material; for aerial, roots, and tissue cultures as mcg./Gm. wet weight. Average of three samples of seeds and aerial portion; and of one sample of roots, callus, and agar medium. [b] Fraction A: alkaline chloroform extract; fraction B: pH 6.8 chloroform extract. [c] Time interval since callus established in culture.

and C_1 were examined for indole alkaloids spectrophotometrically, and A_2, B_2, and C_2 examined for indole alkaloids by thin-layer chromatography.

Quantitative Alkaloid Assay.—Each aliquot was analyzed colorimetrically by the modified *p*-dimethylaminobenzaldehyde (PDAB)–nitrite procedure of Michelon and Kelleher (16). Total alkaloids present were calculated from an ergonovine maleate standard curve which was measured at 590 mμ in a Bausch & Lomb Spectronic 20 spectrophotometer.

Aliquots A_1 and B_1 (in chloroform) were evaporated to dryness in vacuum. The residue of aliquot A_1 was dissolved in 5.0 ml. of 0.2 *N* sulfuric acid, while the residue of aliquot B_1 was dissolved in 10.0 ml. of 5% aqueous tartaric acid. Aliquot C_1 (the filtered aqueous remainder) was analyzed without further purification. However, a bright yellow color in aliquot C_1 prevented its accurate assay, and the data for it are not reported. For each assay 2.0 ml. of extract was added to 2.0 ml. of 0.1% PDAB dissolved in a concentrated sulfuric acid–water solution (1:1, v/v). After 10 min., 0.1 ml. of freshly prepared 0.1% sodium nitrite aqueous solution was added, mixed, and the absorbance recorded. Each assay was repeated three times.

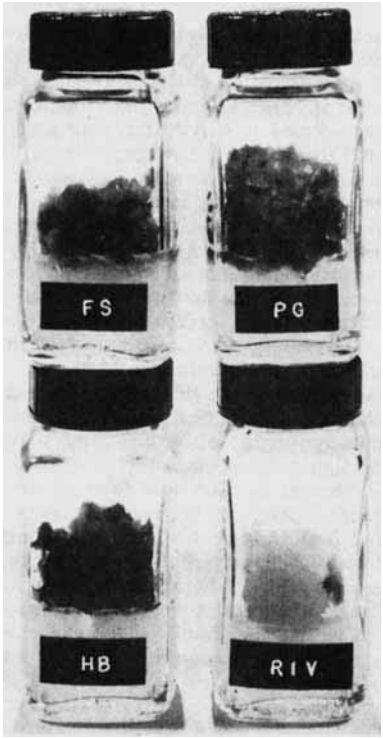

Fig. 1.—Callus tissue cultures after approximately 4 weeks' growth. Key: FS, flying saucer; PG, pearly gates; HB, heavenly blue; RIV, *Rivea corymbosa*.

Qualitative Alkaloid Assay.—Aliquots A_2, B_2, and C_2 were evaporated to dryness in vacuum. The residue of aliquot A_2 was dissolved in 0.5 ml. of 100% ethanol; the residue of aliquot B_2 was dissolved in 0.5 ml. of 95% ethanol; and the residue of aliquot C_2 was dissolved in 1.0 ml. of 95% ethanol. Qualitative comparisons of the alkaloids present in these aliquots were made on thin-layer plates.[2] The plates were spotted with extract amounts ranging from 50 to 200 μl. The solvent system used was a 17:3 mixture of chloroform and methanol (9). Developed plates were sprayed with 1% PDAB in 100% ethanol acidified with concentrated hydrochloric acid, and after 10 min. again sprayed with freshly prepared 0.1% sodium nitrite in 50% ethanol.

RESULTS AND DISCUSSION

Although callus tissue often appeared within 2 weeks on germinated seeds, it required 2 to 3 months before a sufficient size formed for subculture. Once established, callus tissue grew rapidly (Fig. 1) and required subculture approximately every 4 weeks. *Rivea* callus were consistently cream colored, whereas *Ipomoea* callus were considerably darker.

The results of the spectrophotometric assay are shown in Table I, and the chromatographic analysis in Fig. 2. Both analyses reconfirm that fraction A extracts of the seed and aerial portions of *I. violacea* (flying saucer, heavenly blue, and pearly gates) contain alkaloids in the amounts reported by previous investigators (1, 2, 7–9, 11). The authors' results are in agreement with Hofmann (7), but not with Taber *et al.* (9), that alkaloids are present in the plant's roots. Although the three Japanese varieties of *I. nil* seeds assayed contained trace amounts of alkaloids, six domestic varieties are reported not to contain alkaloids (8, 17). Trace amounts of alkaloids remained in most of the alkaline aqueous solutions extracted with chloroform (fraction A). The trace alkaloids were extracted with chloroform

[2] The thin-layer plates were prepared with Adsorbosil-1, Applied Science Lab., Inc., State College, Pa.

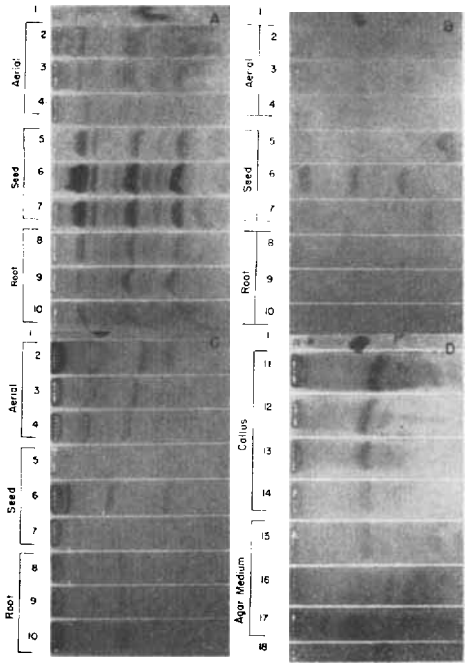

Fig. 2.—Thin-layer chromatograms of morning glory seeds, plants, and tissue cultures. Key: A, plants and seeds: alkaline chloroform extract (fraction A); B, plants and seeds: pH 6.8 chloroform extract (fraction B); C, plants and seeds: pH 6.8 aqueous extract (fraction C); D, tissue culture: alkaline chloroform extract (fraction A). Number code: clymoclavine, 1; pearly gates, 2, 5, 8, 11, 15; heavenly blue, 3, 6, 9, 12, 16; flying saucer, 4, 7, 10, 13, 17; *Rivea corymbosa*, 14; agroclavine, 18. Amounts applied: A, aerial 100 μl.; seed, 50 μl.; root, 150 μl.; B, C, and D, 100 μl. Adsorbent: Adsorbosil-1 (Applied Science Lab., Inc., State College, Pa.). Solvent system: chloroform–methanol (17:3). Spray reagent: PDAB–sodium nitrite.

(fraction B) upon adjustment of the alkaline aqueous solution to pH 6.8 (Table I and Fig. 2, B). The remaining aqueous solution (fraction C) might also contain alkaloids (Fig. 2, C), or indole-type compounds.

Only trace amounts of alkaloids were detected spectrophotometrically in flying saucer, pearly gates, and *Rivea* callus and in flying saucer and pearly gates agar growth medium. No alkaloids were detected spectrophotometrically in heavenly blue tissue cultures (Table I). Although callus tissue cultures more consistently contained alkaloids than their respective medium, the medium extract occasionally gave a more positive and complex pattern than the callus extract on thin-layer plates (Fig. 2, D). Further work is in progress to determine if *Argyreia* and *Ipomoea* suspension cultures produce alkaloids.

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Synthetic Approach to Dihydrokavain

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The preparation of δ-phenylethyl-δ-valerolactone (XII) *via* the alkylation of dihydroresorcinol, followed by a reverse Claisen reaction, and the attempted preparation of dihydrokavain (I), by a modification of this method, are discussed.

FOR CENTURIES the natives of the South Pacific islands have employed the root and rhizome of the kava-kava shrub (*Piper methysticum* Forst.), also known as "ava," "kava," "yangona," and "hoi," to prepare an intoxicating beverage called kava which is consumed at various rituals. The kava beverage, if consumed in sufficient quantity, produces a state of euphoria, followed shortly by muscular relaxation, loss of control of the extremities, and finally a period of dreamless sleep which may last 10 hr. or more. Upon awakening there are apparently no undesirable effects (1–3).

While kava-kava contains a number of components having the pyrone and dihydropyrone

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