Natural Product Letters
Volume 13(3), pp. 233–238
Reprints available directly from the Publisher
Photocopying permitted by license only

INDOLE ALKALOIDS FROM TISSUE-CULTURED *TABERNANTHE IBOGA* H. BN.‡

DOMINICK V. BASILE*[A], MICHELL S. PUNCH[A], JOHN PABLO[B], BRUCE BRENNER[B], W. LEE HEARN[C], AND DEBORAH C. MASH[B]
Department of Biological Sciences, Lehman College of CUNY, Bronx, NY 10468[A] and Department of Neurology, University of Miami Medical School, Miami, FL 3310[B]; Metro-Dade County Medical Examiner Dept., Miami, FL, 33136[C], USA

(Received 24th August 1998)

Abstract:- Tissue lines, selected from explants of *Tabernanthe iboga* H. Bn. and cultured in shake flasks, produced and released from three to five indole alkaloids into the culture medium. The iboga alkaloids in order of their relative abundance were ibogaine, dihydroxyibogamine, ibogamine, voacangine, and ibogaline. All five compounds have the same basic ring structure as ibogaine, a putative anti-addictive drug. Three of these could consistently be detected in culture medium that was removed and replaced at two-week intervals over periods of at least five months. The nutrient-hormone combination used consisted of Gamborg's B5 medium with 2% w/v sucrose, 2mg/l 2, 4-dichlorophenoxy-acetic acid (2,4-D) and 0.1mg/l 6-benzyladenine (BA). These results suggest that plant tissue culture procedures can be developed as an economically feasible and environmentally responsible source of ibogaine and other potential anti-addictive drugs.

Key Words: Ibogaine, tabernanthine, voacangine, ibogamine, dihydroxyibogamine, ibogaline, indole alkaloids, *Tabernanthe iboga*, plant tissue culture.

Tabernanthe iboga H.Bn. is a shrub native to equatorial West Central and West Africa. According to Bisset (1989), different parts or components of the plant (i.e., leaves, roots, latex, and bark,) are used by indigenous peoples of these regions for a variety of purposes. Probably its most widespread use is the chewing of roots or leaves to prevent fatigue and sleep when extended physical effort is undertaken. Warmed leaves are used to treat toothache, the latex as an anthelmentic, and the roots as an anaesthetic and a febrifuge. Some of the compounds produced in the roots and leaves when taken in relatively high doses are hallucinogenic. The principal psychoactive compound produced in the roots is ibogaine, an indole terpenoid.

---

‡ This paper is dedicated to the memory of Ramachandran M.S. Nair, Ph. D who first convinced the primary author of the value of plant tissue culture for obtaining natural products.

Current interest in *T. iboga* alkaloids, especially ibogaine began with anecdotal evidence that ibogaine might prove effective as an anti-addictive drug. For more than a decade, the hypothesis that ibogaine possesses anti-additive properties has been tested in numerous preclinical studies. After reviewing the literature up to 1994, Popik et al. (1995) concluded that, "Ibogaine presents a potential new strategy for treating addiction to diverse drug classes". Literature published subsequently (Glick et al. 1996; Mash et al.1995, 1998), indicates that analogs of ibogaine may equal or surpass ibogaine as an anti-addictive drug.

We have chosen to explore the potential of plant tissue culture techniques for the production of iboga alkaloids. Pawelka and Stöckigt (1983) have already demonstrated that cell suspension cultures of *T. iboga* can produce two indole alkaloids, conoflorine and tubotaiwine. Unfortunately neither had the same basic ring structure as ibogaine.

In the present study, we were able to initiate and select callus cultures that, when incubated in shake cultures, released ibogaine and up to four structurally related iboga alkaloids into the culture medium.

EXPERIMENTAL

Plant material. Stem sections of *T. iboga* from the living plant collection at The New York Botanical Garden served as the starting material. After washing them with hand soap and water, 11-12mm long sections were placed in 6 x 13 cm screw-capped jars, immersed in 95% ethanol for 1-2min, followed by immersion in 20% v/v aqueous Clorox™ solution containing 0.1% wt/v Alconox™ detergent for 30 min with occasional shaking, and finally rinsing them in three, 1-2 min changes of sterile, distilled water. The stem sections were transferred to sterile plastic Petri dishes and cut into segments of about 15mm. A single stem segment was used to inoculate each culture vessel used to induce callus formation.

Culture conditions. Callus cultures were initiated and maintained as stock cultures in "baby food jars" (Sigma-Aldrich) containing 20ml heat sterilized B5 medium (Gomborg et al. 1968), supplemented with 2mg/l 2,4-D, 0.1 mg/l BAP and 2 % wt/v sucrose. Media for stock cultures was solidified with 4.6 g/l Gellan (Research Organics). Magenta Caps™ (Sigma-Aldrich) were used as closures. Stocks were subcultured every 4-6 weeks, depending on their relative rates of growth. To test for alkaloid production, shake cultures were initiated from stock callus cultures. Shake cultures consisted of 1 liter DeLong® culture flasks with clear plastic closures (Bellco Glass) containing 300ml heat-sterilized aqueous medium of the same composition as the stock cultures and inoculated with an average of 25 g (21-33g) fresh wt callus tissue. The flasks were shaken on gyrotory shakers (New Brunswick G-10) set for 125 rpm. Both stock and shake

cultures were incubated in a temperature-controlled room maintained at 25 ± 3°C. Fluorescent lamps over both stock and shake cultures furnished continuous light at shelf or platform level of 650 –1000 lux.

Assay procedures. Only the aqueous medium from the shake cultures was assayed for alkaloids. Every two weeks, the medium from each of the shake cultures was carefully decanted into clean beakers (after the suspended cells and cell masses were allowed to settle) and replaced by 300 ml of freshly prepared medium. The "spent" medium was filtered through Whatman # 4 paper to remove the cells/cell masses that remained suspended. The filtrate was then passed through a C18 SPE column to retain the alkaloids that were subsequently eluted with methanol. The eluates were taken to dryness under reduced pressure and resuspended in methanol. TLC on 4 x 8 cm Polygram® Sil G/UV plates (Alltech) was used to check for the presence of alkaloids in the extracts. They were developed with methanol: 1% aqueous formic acid (6:4) and sprayed with Dragendorff's Reagent to detect the presence of alkaloids.

Quantitative gas chromatography/mass spectroscopy (GC/MS). The iboga alkaloids were extracted from the SPE column eluates by means of solvent extraction under basic conditions as described by Hearn et al. (1995). The GC/MS was operated in the full scan electron ionization mode scanning from M/Z 45 to 450at 1sec/scan. Compound identification was based on comparison of retention times and fragmentation patterns obtained from authentic standards (s.a. Omnichem, Belgium). The limits of detection and quantitation were 0.5ng/ml.

RESULTS AND DISCUSSION

It was difficult to establish an adequate supply of axenic callus cultures to use as a source of inoculum for shake cultures due to an initially high percentage of contamination, from apparently endogenous fungi and bacteria. However, when freed from contaminants, the nutrient-hormone combination chosen on the basis of past experience (Pawelka and Stöckigt 1983, Basile et al 1993) proved sufficient to initiate and maintain callus cultures. The appearance of the calli when first initiated varied in color from deep green to light tan and in texture from hard and compact to soft and friable. Experiments to correlate phenotypic differences with differences in alkaloid production indicated that the light tan friable callus tissue produced the most Dragendorff-positive compounds. Therefore, cultures showing this phenotype were chosen for establishing sufficient stock cultures needed to inoculate shake cultures.

Three sets of five replicate shake cultures were initiated at irregular intervals, but were tested for alkaloid production at regular two-week intervals, thereafter. TLC analysis of the pooled media from each set of replicate cultures revealed the presence of from three to five Dragendorff's Reagent-positive spots. No

qualitative changes in alkaloid produced in a set of shake cultures could be detected by TLC analysis in experiments conducted for periods up to six months. When samples from the set of cultures that appeared to contain the most alkaloids were analyzed by GC/MS, five iboga alkaloids were identified. These were ibogaine, dihydroxyibogamine, ibogamine, voacangine, and ibogaline (Fig. 1.). All five have the same basic ring structure. Ibogaine (12-hydroxyibogamine) was confirmed to be the compound responsible for the mass spectrum with molecular ion m/z 310 as apposed to tabernanthine (13-hydroxyibogamine, a positional isomer) by relative chromatographic retention times using standards of ibogaine and tabernanthine (data not shown). For dihydroxyibogamine (Fig. 1B) the mass spectrum reflects two hydroxyl groups potentially bound in any combination between positions 11-14. However, the structure demonstrated has hydroxyl groups at positions 12 and 13 analogous to the methoxy groups of ibogaline and likely represents the natural conformation for the plant. The relative abundance of the iboga indole–like compounds in the extract, based on percent contribution of each total ion peak area versus sum of these areas, was 41.7, 27.0, 22.9, 6.9, and 1.4%, respectively. When media extracts yielding only three spots detected by TLC were analyzed by GC/MS, only ibogaine, voacangine and ibogamine were detected with a relative abundance of 42.9, 35.7 and 21.5%, respectively.

The goal of this study was to find out if cell suspension cultures derived from *Tabernanthe iboga* H. Bn. can be induced to produce the same compounds normally synthesized in the roots of intact plants. We were particularly interested in determining conditions that will result in the production of the ibogaine and associated precursor compounds that are currently being investigated as potential anti-addictive drugs (Mash et al. 1995, 1998; Glick et al. 1996, Popik et al. 1995). In an earlier study, Pawelka and Stöckigt (1983) reported that suspension cultures of *T. iboga* produced only two iboga alkaloids, neither one of which has the same ring structure as ibogaine. The present study yielded significantly different results. The five iboga alkaloids identified from our suspension cultures have the same ring structure as the putative anti-addictive compound, ibogaine. Furthermore, the cultures did not have to be sacrificed in order to obtain the indole alkaloids. These results are analogous to those whereby cultured tissues of *Artemisia annua* L. were induced to produce and release artemisinin, an antimalarial/antiparasitic compound, into the culture medium (Basile et al 1995). The culture conditions and procedures developed thus far resulted in the consistent and stable production of from three to five iboga alkaloids extractable at two-week intervals over periods of several months. At present, the maximum duration that sets of cultures would continue to produce iboga alkaloids is not determined, but there was no obvious change in product yield as long as the culture medium was replenished at regular two-week intervals. It is not realistic at this stage of development to determine quantities/"harvest" or to estimate annual yields for selected compounds. The procedures used in this study were unsuitable to large-scale production or the accurate determination of yield relative to cell mass. Neither the amount of tissue produced during each two weeks of incubation nor the amount of tissue lost each time the medium was decanted from a flask could be reliably

determined. Nevertheless, based on these initial results, we think it reasonable to expect that by scaling up from shake flasks to bioreactors and further adjusting culture conditions it may be possible to significantly increase the production of iboga alkaloids. The fact that we are already able to obtain from three to five compounds having the same basic ring structure, as ibogaine is most encouraging. Evidence is accumulating that derivatives of ibogaine may prove to be safer, if not more effective as interrupters of drug addiction (Mash et al. 1995, 1998 Glick et al. 1996). The results reported here suggest that the compounds already being produced by the cultures could be used as starting materials for the custom synthesis of ibogaine analogs. These results also suggest that the large-scale culture of *Tabernanthe iboga* cell/tissue lines we already have in culture may provide an economically feasible source of lead compounds for the development of safe and effective anti-addictive drugs.

ACKNOWLEDGMENT: This research was supported in part by NIH Grant 5S06GM08225-10.

REFERENCES

1. D. V. Basile, N. Akhtari, Y. Durand, et al. (1993) Toward the production of artemisinin through tissue culture: Determining nutrient –hormone combinations suitable for cell suspension cultures. *In Vitro Cell Devel Biol.*, **29P**, 143-147.
2. M.R. Basile, N. Akhtari, et al. (1995) Toward optimizing the nutrient-hormone composition for the production of artemisinin by Artemisia annua. In *Recent Discoveries in Natural Products Chemistry*. eds. (A-u Rahman, M.I. Chaoudhary, and M.S. Shekhani) Elite Publishers, Karachi, Pakistan.
3. N.G. Bisset (1989) Tabernanthe: uses, phytochemistry, and pharmacology. *Wagengen Agric. Univ. Papers* **89**, 20-26.
4. O.L. Gamborg, R.A. Miller, and K. Ojima (1968) Nutrient requirements of suspension cultures of soybean root cells. *Exp. Cell Res.* **50**, 151-158.
5. Glick, S.D., Kuehne, M.E. et al. (1996) 18-methoxycoronaridine, a nontoxic iboga alkaloid congener: effects on cocaine and morphine self administration and on mesolimbic dopamine release in rats. *Brain Res.* **719**, 29-35.
6. W.L. Hearn, J. Pablo, et al. (1995) Identification and quantification of ibogaine and an O-demethylated metabolite in brain and biological fluids using gas chromatography mass spectroscopy. *J. Anal. Toxicol.* **19**, 427-434.
7. K.H. Pawelka, and J. Stöckigt (1983) Indole alkaloids from suspension cultures of *Tabernaemontana devaricata* and *Tabernanthe iboga*. *Plant Cell Rep.* **2**, 105-107.
8. P. Popik, R.T., Layer, and P. Skolnick (1995) 100 Years of ibogaine: neurochemical pharmacological actions of a putative anti-addictive drug. *Pharmacological Reviews* **47**, 235-253.

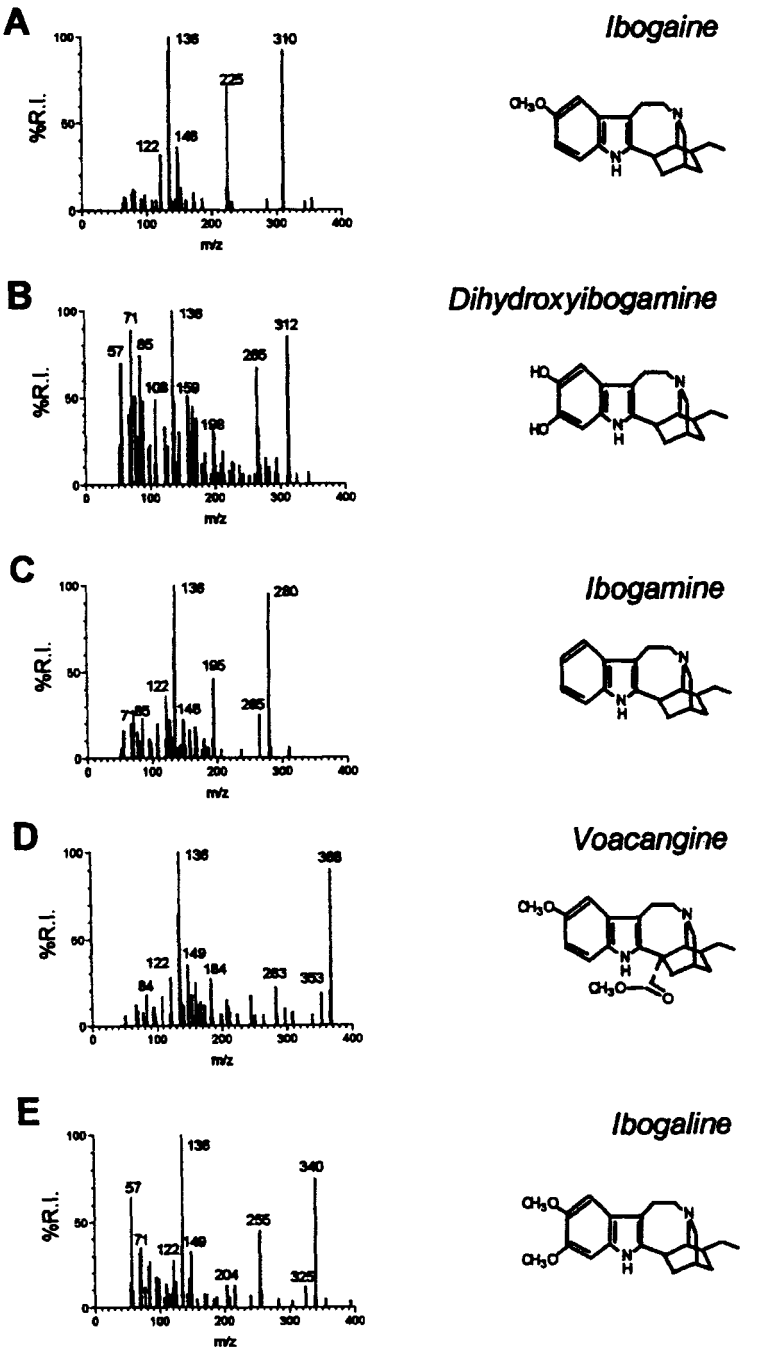

Fig. 1. Full scan electron impact mass spectra from extracted cell culture supernatant. Mass spectra of (A) ibogaine (m/z 310), (B) dihydroxyibogamine (m/z 312), (C) ibogamine (m/z 280), (D) voacangine (m/z 368), and (E) ibogaline (m/z 340) taken from individual chromatographic peaks.