of these ergot alkaloids, and little attention is being given to procedures for their chemical identification and quantitation. Recent studies suggest that subclinical and combined toxicities of alkaloids may be a more insidious detriment to both livestock and human health than the individual compounds (Davis et al., 1983; Porter et al., 1983, 1985). Although extensive biological data exist on the ergot peptide and clavine alkaloids (Berde and Schield, 1978), little is known concerning their synergistic and/or subclinical toxicities as they exist in nature.

ACKNOWLEDGMENT

We thank L. K. Tate, G. M. Adcock, and J. C. Herndon for technical assistance during the course of this study.

**Registry No.** Ergotamine, 113-15-5; ergosine, 561-94-4; β-ergosine, 60192-59-8; ergonine, 29537-61-9; ergovaline, 2873-38-3; ergostine, 2854-38-8; ergoptine, 29475-05-6; β-ergoptine, 65756-55-0; ergocornine, 564-36-3; ergocristine, 511-08-0; ergocryptine, 511-09-1; β-ergocryptine, 20315-46-2.

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Received for review September 2, 1986. Accepted February 9, 1987.

# Indolealkylamines of *Desmanthus illinoensis* and Their Growth Inhibition Activity

Alonzo C. Thompson,* Gilles F. Nicollier,[1] and Daniel F. Pope

Methanol extracts of *Desmanthus illinoensis* roots inhibited the growth of tomato and radish seedling roots. Indole-3-aliphatic acids caused measurable root growth inhibition at 0.1 and 1.0 ppm, and the activity increased as the acid side chain decreased. Indole-2-carboxylic acid did not show significant activity. *N*-Hydroxy-*N*-methyl-1*H*-indole-3-ethanamine and 2-hydroxy-*N*-methyltryptamine were isolated and identified by $^1$H NMR and MS/CI. Structural confirmation for these compounds was obtained with ammonia and deuteriated ammonia MS/CI.

*Desmanthus illinoensis* (Michaux), a legume frequently found growing in disturbed fields and roadsides in the southern part of the United States, was collected in Oktibbeha County, MS, and identified by the Botanical Institute at Mississippi State University. Previously, we reported the isolation, identification, and phytotoxic properties of the flavonoids from the leaves of this plant (Nicollier and Thompson, 1983). Nicollier et al. (1984) found phytotoxicity in the total root extracts of this plant.

## MATERIALS AND METHODS

**Extraction of *D. illinoensis*.** Plant roots were washed and air-dried. The bark (378 g) was separated from the woody roots (350 g), and both were pulverized and extracted with 2.5 L of methanol. After filtering, the residue was refluxed in methanol for 2 h and filtered. Cold (room temperature, 24 h) and hot extractions were made of each filtrate. After TLC and HPLC showed no differences, the hot and cold extracts of bark and roots were combined to

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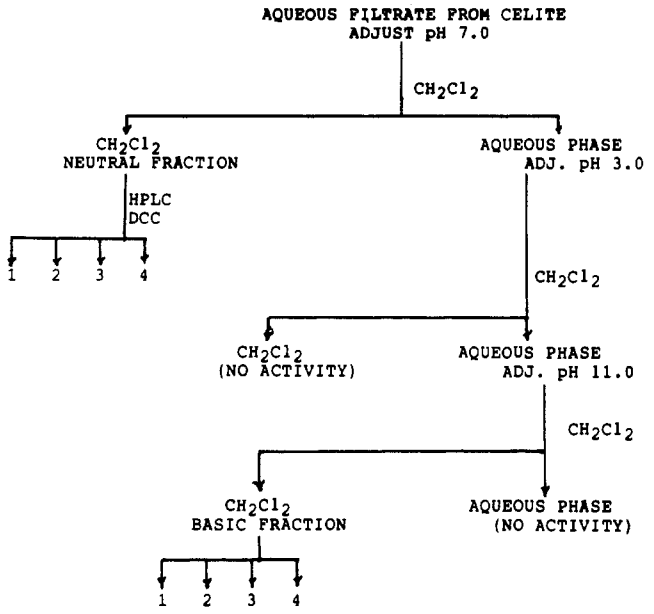


**Figure 1.** Fractionation of the methanolic extract of *D. illinoensis* roots and bark after washing through Celite with water.

give a single solution of each. Each extract was concentrated at 45 °C under reduced pressure. The extracts were filtered through Celite to remove the precipitated chlorophyll and fractionated according to the scheme of Figure 1 into neutral, acidic, and basic soluble fractions.

**Chromatography.** The neutral (3.74-g) and basic (1.44-g) fractions of bark and the neutral (1.04-g) and basic (0.07-g) fractions from the root were analyzed individually by silica gel TLC. Separation of the mixtures was made by DCC (droplet countercurrent apparatus, Tokyo Rikakikai, Nishikawa, Tosyama-cho, Kanda Chiyda-ku, Tokyo, Japan) with the solvent system $CHCl_3$–MeOH–PrOH–HOH (5:6:1:4, v/v/v/v). The upper phase of the solvent system was used as the stationary phase and the lower phase as the moving phase. The eluates were further separated by HPLC using Partisil-10, C-8 column (Whatman; 50 cm, i.d. 4.6 mm) in the solvent system AcOEt–*i*-PrOH–$NH_4OH$ (43.5:35:1, v/v/v).

**Mass Spectral Characterization.** Mass spectra were obtained with a Hewlett-Packard 5985B unit equipped with dual EI/CI source. Chemical ionization (CI) spectra were first obtained with methane ($CH_4$) as the ionization gas, followed by ammonia ($NH_3$) and deuteriated ammonia ($ND_3$). The ammonia gases were used at an ion-source pressure of 0.2 Torr at 150 °C.

Previously, Buchanan (1982) investigated the degree of substitution on amino groups by GC–MS of a complex mixture derived from coal tar. We modified her procedure by employing direct-probe MS using ammonia and its deuteriated counterpart.

**Bioassay.** Each compound to be tested was dissolved or dispersed in distilled water at a concentration of up to 100 ppm. Three 10-$cm^2$ pieces of germination paper were placed in a 10-$cm^2$ plastic disposable petri dish. The paper was moistened with 5 mL of the appropriate test solution. Five petri dishes were used in each test. Twenty-five seeds of tomato (*Lycopersicon eseulentum*, Miller cv. Homestead 24) and radish (*Raphanus sativus* L. cv. Champion) were equally spaced on the germination paper in separate dishes and incubated at 10 °C. Radish root length was measured after 96 h in the dark and tomato after 186 h. The roots were measured to the nearest millimeter and the mean length and standard deviation determined and compared to a water control.

**Table I. Differentiation of Amines by CI/MS Using Protiated and Deuteriated Ammonia as Ionization Gases**

| compd | molecular weight | | |
|---|---|---|---|
| | normal | $NH_3$ (CI) | $ND_3$ (CI) |
| aniline | 93 | 94 | 97 |
| *N*-ethylaniline | 121 | 122 | 124 |
| *N,N*-dimethylaniline | 121 | 122 | 123 |

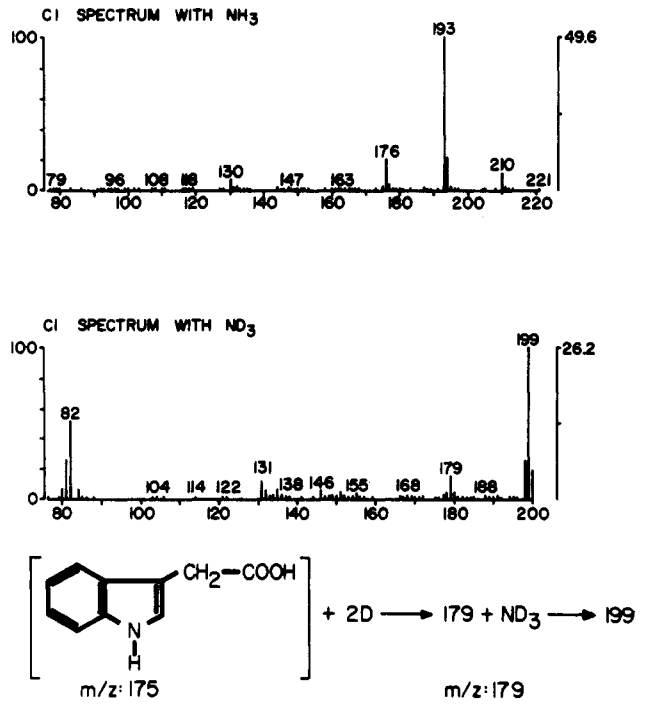


**Figure 2.** MS/CI of indole-3-acetic acid.

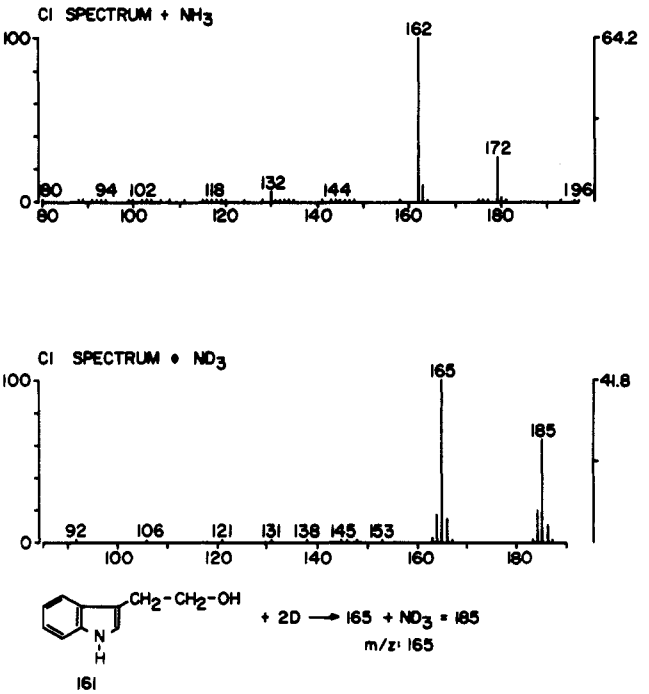


**Figure 3.** MS/CI of tryptophol.

## RESULTS AND DISCUSSION

**Neutral and Basic Compounds.** When ammonia is used to differentiate amines by MS/CI, the amines are ionized by transfer of hydrogen ion from the ammonia to the amine (Table I):

$$R_{3-n}NH_n + NH_4^+ \rightarrow [R_{3-n}NH_{n+1}]^+ + NH_3$$

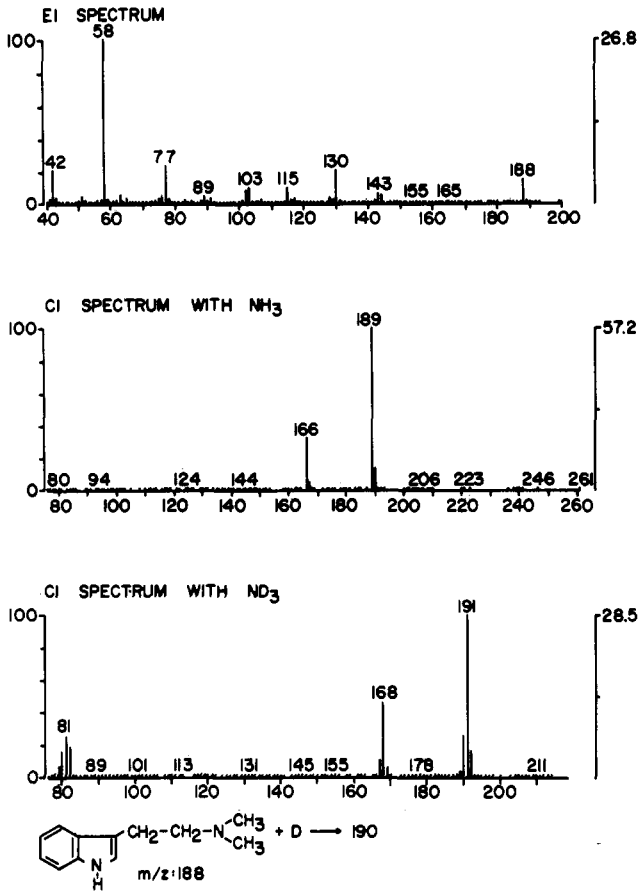


**Figure 4.** MS/EI and MS/CI of *N,N*-dimethyltryptamine.

If ammonia-$d_3$ is used, amines are ionized by transfer of a deuterium and the amine hydrogen transfer to the ammonia:

$$R_{3-n}NH_n + ND_4^+ \rightarrow [R_{3-n}ND_{n+1}]^+ + NH_nD_{3-n}$$

Figures 2–4 give the MS/CI spectra of indole-3-acetic acid, tryptophol, and *N,N*-dimethyltryptamine isolated from *D. illinoensis*. When deuteriated ammonia is used, all of the labile protons of the oxygen and nitrogen are replaced by deuterium. *N,N*-Dimethyltryptamine (Figure 1, fraction 1) was identified from the neutral and basic fractions and constitutes 2% and 25% of the total extract in the root and root bark, respectively. The compound was characterized by MS (EI/CI, Figure 4), proton and $^{13}C$ NMR, and thin-layer cochromatography with known reference compounds. *N*-Methyltryptamine was present in the root bark and root at 8% and 0.5% of total extract, respectively. Gramine (Figure 1, neutral fraction 2) was isolated in low concentration from the neutral fraction of root bark. Identification was made by the same methods used for the methyl-substituted tryptamines.

Fraction 3 from the DCC chromatography of the neutral fraction (Figure 1) gave 20 mg of a compound producing the same color reaction with vanillin–sulfuric acid reagent as the tryptamines: $^1H$ NMR (80 MHz, $CDCl_3$) $\delta$ 9.0–9.3 (br s, 1 H, 1H-indole), 7.6–6.8 (m, 5 H, H-2, H-5–8), 3.71–3.3 (m, 4 H, $CH_2CH_2$), 3.2 (s, 3 H, $NCH_3$), 1.5 (s, 1 H, NH). The shifts for the protons of the indole portion of the molecule are similar to those encountered for *N*-methyltryptamine, except that the two methylene groups appear as a multiplet due to the different substituents on the nitrogen. The methylene groups appear as a singlet in *N*-methyltryptamine. Also, the singlet for the $NCH_3$ is shifted downfield due to the OH substituent on the nitrogen. The compound had the following mass spectral

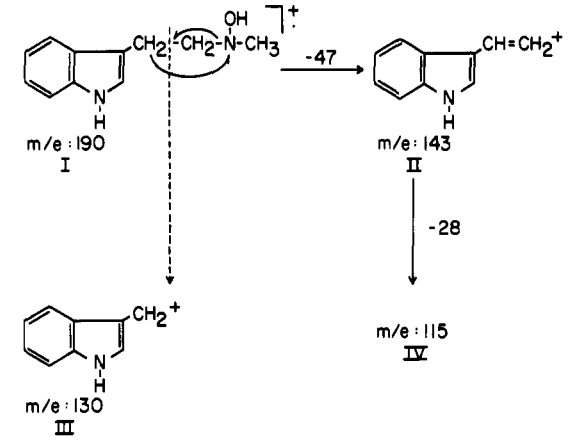


**Figure 5.** Partial fragmentation pattern of MS/EI of *N*-hydroxy-*N*-methyl-1*H*-indole-3-ethanamine.

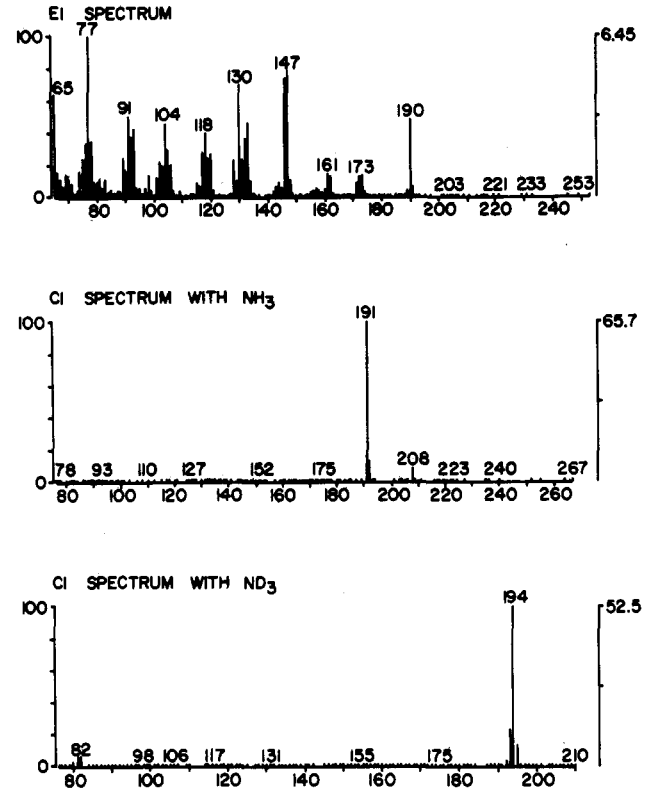


**Figure 6.** MS/EI and MS/CI of 2-hydroxygramine.

properties: MS/EI *m/z* (rel intens) 190 (60, $M^+$), 143 (100), 130 (45), 115 (42), 93 (20), 77 (30); MS/CI (methane) *m/z* 191 (10), 144 (100); MS/CI ($ND_3$) *m/z* 194 (100), 155 (60), 146 (50). From these data, this compound was assigned the structure *N*-hydroxy-*N*-methyl-1*H*-indole-3-ethanamine (Figure 5, structure I). The major fragment (Figure 5) is produced by the loss of the $N(OH)CH_3$ (47 amu) to give the *m/e* 143 base ion. The direct removal of the methylenehydroxymethylamine gives the less stable 3-methyleneindole fragment (*m/e* 130).

Fraction 4 from the DCC separation of the neutral fraction (Figure 1) gave a compound (50 mg) that gave the same color with vanillin–sulfuric acid reagent as the other tryptamines. The compound had an IR absorption at 744 $cm^{-1}$ characteristic of four adjacent hydrogens in the indoles. However, there were strong bands at 3300 and 1570–1550 $cm^{-1}$ that do not occur in other indolealkylamine spectra: $^1H$ NMR (80 MHz, $CDCl_3$) $\delta$ 7.24–7.11 (m, 2 H, H aromatic), 6.77–6.64 (m, 2 H H aromatic), 4.42–4.40 (br s, 1 H, OH), 3.89–3.80 (br s, 2 H, m, $CH_2$), 2.43–2.40 (m, 2 H, $CH_2$), 2.39 (s, 3 H, $CH_3$). In deuteriated water, the

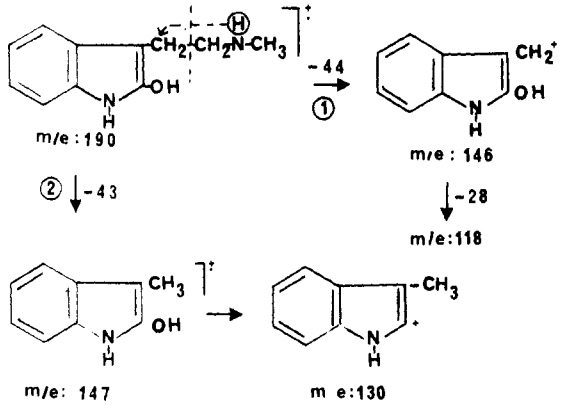


**Figure 7.** Partial MS/EI fragmentation pattern of 2-hydroxy-*N*-methyltryptamine.

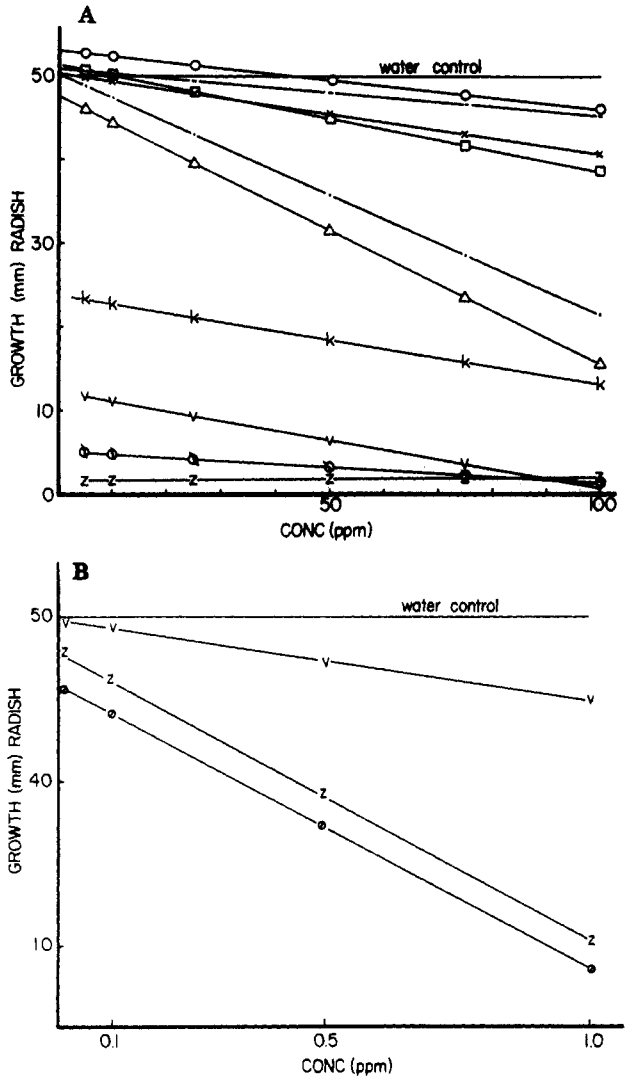


**Figure 8.** Linear regression of the effect of concentration of indoles on the growth of radish seedlings. Key: indole-3-acetic acid (○); 5-hydroxyindole-3-acetic acid (---); indole (△); 2,3-dimethylindole (×); 5-hydroxyindole (-·-); 5-methylindole-3-acetic acid (z); indole-3-butyric acid (v); tryptophol (k); 1,3-dimethylindole (⊖); indole-2-carboxylic acid (□).

signals at δ 4.42 and 3.898–3.80 are shifted. The major difference between this NMR spectrum and those of the other indolealkylamines is the presence of the hydroxyl group and the symmetry of the two sets of aromatic protons, which indicates a modification in the adjacent phenyl ring. Figure 6 gives the MS/EI and MS/CI of this compound and Figure 7 the partial MS/EI fragmentation. The

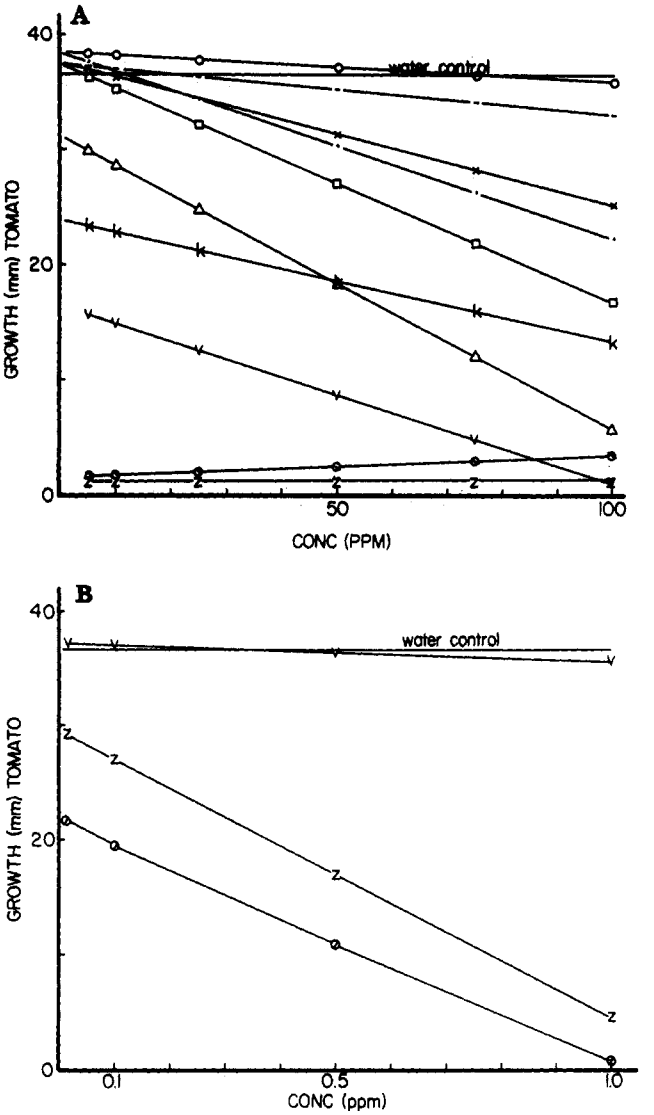


**Figure 9.** Linear regression of the effect of concentration of indoles on the growth of tomato seedlings (legends same as in Figure 8, parts A and B).

compound was identified as 2-hydroxy-*N*-methyltryptamine. Chromatography of the basic fraction gave the same compounds as found in the neutral fraction.

**Phytotoxic Properties of the Indolealkylamines.** Parts A and B of Figure 8 give the root growth inhibition of radish seedlings at various concentration levels of indole derivatives isolated from the root bark of *D. illinoensis*. These linear regressions are not intended to show the best curve fit for the data but to show general differences in the activity of the compounds assayed compared to the water control. The activity of the substituted indole acids was too great to measure at concentration levels of 0–100 ppm, so they were therefore evaluated at concentrations of 0.1–1.0 ppm (Figure 8B).

The same general pattern of root growth inhibition by the indole derivatives was shown toward tomato (Figure 9A,B) as that observed for radish. Even though indole-3-butyric acid was strongly inhibitory to tomato and radish root growth at high levels (0–100 ppm), it was not significantly different from the water control at 0.1–1.0 ppm.

ACKNOWLEDGMENT

We are grateful to M. Burks for mass spectral data and P. A. Rebetez for his technical support.

**Registry No.** Indole-3-acetic acid, 87-51-4; 5-hydroxyindole-3-acetic acid, 54-16-0; indole, 120-72-9; 2,3-dimethylindole, 91-55-4;

5-hydroxyindole, 1953-54-4; 5-methylindole-3-acetic acid, 1912-47-6; indole-3-butyric acid, 133-32-4; tryptophol, 526-55-6; 1,3-dimethylindole, 875-30-9; indole-2-carboxylic acid, 1477-50-5; *N,N*-dimethyltryptamine, 61-50-7; *N*-hydroxy-*N*-methyl-1*H*-indole-3-ethanamine, 57383-99-0; 2-hydroxy-*N*-methyltryptamine, 106987-89-7; gramine, 87-52-5.

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Received for review June 5, 1986. Revised manuscript received August 28, 1986. Accepted December 5, 1986. Mention of a trademark proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

# Modification of Red Squill by *Aspergillus niger*

Anthony J. Verbiscar,* Thomas F. Banigan, and Robert A. Schatz

Five strains of *Aspergillus niger* produced a β-glucosidase that cleaved scilliroside to its aglycon scillirosidin. After incubation for 6 days in submerged cultures, yields of scillirosidin for each strain ranged from 23 to 100%. Red squill bulb extracts were treated with *A. niger* and naringinase, a mixed-function enzyme derived from *A. niger*. Aglycons were isolated and showed a toxicity to rats that parallels their scillirosidin content. The scillirosidin aglycons are more toxic to female rats than to males.

Red squill (*Urginea maritima, lilliaceae*) has been used as a rodenticide since the Middle Ages (Chitty, 1954). The bulbs and roots contain scilliroside and other toxic scilla glycosides and are generally formulated into rodent baits as dried powders. The toxicity of the dried bulb powders varies substantially due to genetic variants in the wild seed propagated plants, differences in harvest time, and decomposition on storage (Verbiscar et al., 1986; Gentry et al., 1987). Bioavailability may also affect toxicity. The dry powders are composed of hard mucilagenous granules from which the scilla glycosides may not be readily extracted into rodent stomach fluids, and hence not absorbed. Solvent extracts of red squill have been used in rat baits in an attempt to solve these problems. However, the extracts of scilla glycosides are extremely bitter, and rodents quickly learn to avoid such baits. The extracted glycosides are more readily available to taste receptors in the rodent palate than are the glycosides in dry powders. The utility of red squill preparations for rodent control is related to acceptability as well as toxicity.

A goal of this study was to convert the scilla glycosides in red squill to their aglycons, thereby increasing palatability. Scilliroside, the major toxic glycoside in red squill, is bitter, whereas its aglycon scillirosidin is tasteless. Scillirosidin is equally or more toxic than the parent glucoside (Rothlin and Schalch, 1952; Stoll and Renz, 1950). Chemical methods designed to cleave the glucose from scilliroside are too drastic, causing hydrolysis of the lactone and acetyl grops (von Wartburg and Renz, 1959), resulting in decreased toxicity (Verbiscar et al., 1986). Accordingly, enzymatic methods were chosen for this conversion.

Free scillirosidin has been detected in some bulb clones, but red squill does not contain the enzyme necessary to cleave glucose from scilliroside. Commercial β-glucosidase from bitter almonds does not cleave this glucoside (Stoll and Renz, 1942; Verbiscar et al., 1986). The enzymatic conversion of scilliroside to scillirosidin was successful on extracts of the seeds from *Coronilla glauca* and *Medicago sativo* (Stoll and Renz, 1950). Scilliroside has also been cleaved by enzymes in fungi including *Penicillium sp.*, *Aspergillus sp.*, and others (Stoll et al., 1951a,b). Naringinase, a mixed-function enzyme from *Aspergillus niger*, cleaves first rhamnose and then glucose from naringen (Horowitz, 1981). We found that naringinase cleaved the glucose from scilliroside, desacetylscilliroside, and scillaren A, providing scillirosidin, desacetylscillirosidin, and proscillaridin, respectively (Verbiscar et al., 1986). With this basis, five strains of *Aspergillus niger* were screened for their ability to produce a β-glucosidase that would cleave scilliroside to its aglycon (Figure 1).

Anver Bioscience Design, Inc., Sierra Madre, California 91024 (A.J.V., T.F.B.), and College of Pharmacy and Allied Health Professions, Northeastern University, Boston, Massachusetts 02115 (R.A.S.).

## EXPERIMENTAL SECTION

**Materials.** Five strains of *A. niger* (NRRL 3, 330, 337, 372, 6411) were obtained from the Culture Collection, Northern Regional Research Laboratory, U.S. Department of Agriculture, Peoria, IL, courtesy of C. W. Hesseltine. Naringinase derived from *A. niger* was obtained from Sigma Chemical Co. Red squill bulbs were provided courtesy of H. S. Gentry, Gentry Experimental Farm, Murrieta, CA. Malt extract broth was obtained from Becton Dickenson and peptone from Difco.

**Culture Conditions.** The *A. niger* strains were maintained on slants of Blakeslee's formula consisting of 5 g of malt extract broth, 5 g of dextrose, 5 g of agar, and 250 mg of peptone brought to 250 mL with distilled water. Submerged cultures were carried out in Czapek Dox broth (Difco Manual, 1953) containing 30 g of sucrose, 3 g of sodium nitrate, 1 g of dipotassium phosphate, 0.5 g of magnesium sulfate, 0.5 g of potassium chloride, and 0.01 g of ferrous sulfate made up to 1 L with distilled water. Media were autoclaved for 15 min at 121 °C. Milk filter disk closures were used for the *Aspergilli*, which were incubated at 30 °C for propagation and growth. A gyratory shaker at 150–200 rpm was used to increase aeration, growth rate, and scilliroside hydrolysis rate. The propagation slants were stored refrigerated at 8 °C.

**Analytical Methods.** Thin-layer chromatography (TLC) was run on Merck silica gel G using various de-

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