Ergobalansine, a New Ergot-Type Peptide Alkaloid Isolated from Cenchrus echinatus (Sandbur Grass) Infected with Balansia obtecta, and Produced in Liquid Cultures of B. obtecta and Balansia cyperi

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ERGOBALANSINE, A NEW ERGOT-TYPE PEPTIDE ALKALOID ISOLATED FROM *CENCHRUS ECHINATUS* (SANDBUR GRASS) INFECTED WITH *BALANSIA OBTECTA*, AND PRODUCED IN LIQUID CULTURES OF *B. OBTECTA* AND *BALANSIA CYPERI*[1]

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ABSTRACT.—An EtOH extract of *Cenchrus echinatus* (sandbur grass) infected with *Balansia obtecta*, a fungal endophyte of the Clavicipitaceae, has yielded ergobalansine [**1**], a new ergot-type alkaloid. Ergobalansine is a peptide derivative of lysergic acid, but differs from other known ergopeptine alkaloids in that the characteristic proline residue has been replaced by an alanine residue. The structure of ergobalansine was determined by analysis of ^{1}H nmr, ^{13}C nmr, and mass spectra of the parent compound and of the C-8 epimer (ergobalansinine [**2**]) and by comparison of these to spectra of the common ergopeptine alkaloids.

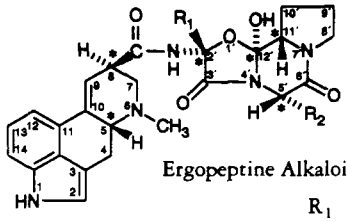
Cenchrus echinatus L. (Gramineae) is an annual grass species native to the tropics or subtropics. It ranks highly among the world's worst weeds and has been reported as a serious pest in at least 18 crops in 35 countries (1). In the United States, *Ce. echinatus* is common along the South Atlantic and southern border area (2). *Ce. echinatus* is often referred to as sandbur, burgrass, or sandspur. When present as contaminants in feeds and hay, the burs of the seed heads reduce its acceptability and palatability for animals. Sandbur grass grows rapidly in sandy well-drained soils and often is found in cultivated fields, pastures, abandoned fields, and lawns, along roadsides, and along beaches. A search of the literature has revealed that there have been no detailed studies of the secondary metabolites of sandbur grass.

Fungal endophytes of grasses, and ergot-like toxicity associated with endophyte-infected grasses, have become important topics during the last decade (3–5). *Acremonium coenophialum* and *Acremonium lolii*, the endophytes of tall fescue and perennial ryegrass, respectively, have received considerable attention, as these organisms are responsible for substantial losses to cattle producers in the United States and New Zealand. Ergovaline, one of the typical ergopeptine alkaloids long known to occur in *Claviceps* species (Figure 1) (6), is clearly a factor in the toxicity associated with tall fescue (7). Several other forage grasses, and a number of weed grasses and sedges, serve as hosts for the 13 *Balansia* species (Clavicipitaceae) known to be endemic to the United States (3). *Balansia* species have been reported to produce ergot alkaloids such as the clavines and ergonovine (3,8) in liquid culture, and *Balansia*-infected grasses have been observed to produce ergotism-like symptoms in cattle; however, there are no reports of ergopeptine alkaloid production by *Balansia* species. In particular, cultures of *Balansia obtecta* Diehl, parasitic on *Ce. echinatus*, and *Balansia cyperi* Edgerton, parasitic on *Cyperus virens* Michx., failed to produce alkaloids in a semisynthetic liquid medium (8).

Fungal-infected sedges and grasses are often more resistant to attack by insect herbivores than are the corresponding plants that are not infected (5,9,10), and certain al-

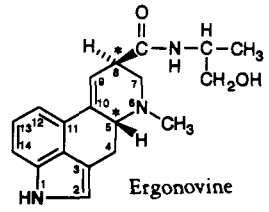
[1]The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

[2]USDA/ARS, Retired.



Ergopeptine Alkaloids

	R_1	R_2
Ergotamine	Me	$PhCH_2$
Ergosine	Me	i-Bu
β-Ergosine	Me	sec-Bu
Ergovaline	Me	i-Pr
Ergostine	Et	$PhCH_2$
Ergoptine	Et	i-Bu
β-Ergoptine	Et	sec-Bu
Ergonine	Et	i-Pr
Ergocrystine	i-Pr	$PhCH_2$
α-Ergokryptine	i-Pr	i-Bu
β-Ergokryptine	i-Pr	sec-Bu
Ergocornine	i-Pr	i-Pr



Ergonovine

FIGURE 1. The common ergopeptine alkaloids consist of D-lysergic acid linked to a tricyclic peptide moiety and have six centers of asymmetry as indicated by asterisks. Ergonovine is one of the better known amides of lysergic acid. The corresponding C-8 epimeric compounds are named with an additional "in"; for example, the C-8 epimer of ergotamine is ergotaminine.

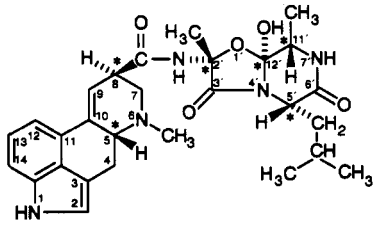
kaloids known to be present only in fungal-infected plants exhibit insect toxicity or antifeedant activity (11,12). Our long-term interest in cattle production problems associated with ingestion of endophyte-infected tall fescue, our more recent interest in the causes of insect resistance of fungal-infected plants, and the availability of instrumentation required for specific detection of ergopeptine alkaloids present in complex mixtures at low concentrations (13,14) prompted us to initiate the present study.

RESULTS AND DISCUSSION

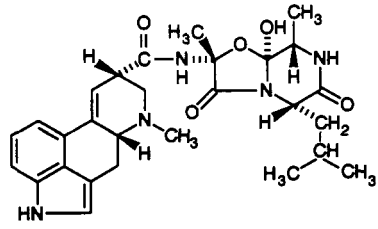
Initial examination of culture extract of *B. obtecta* and *B. cyperi* and extracts of *C. echinatus* (infected with *B. obtecta*) and *C. virens* (infected with *B. cyperi*) for the presence of ergopeptine alkaloids (13,14) indicated that all of these materials contained one or more compounds that behaved as typical peptide derivatives of lysergic acid. However, the compound(s) observed did not correspond to any lysergic acid peptides previously described in the literature. Selection of *C. echinatus* for fractionation, isolation, and ultimate characterization of the unknown compound(s) was based on observations that infected *C. echinatus* contained the major unknown at a concentration greater than 1 ppm; also, the plant material was available in quantity. Fractionation of an EtOH extract of *C. echinatus* by hplc on silica, concentration of alkaloids by solvent partitioning between

EtOAc and aqueous acid, extraction of alkaloids into EtOAc (after basification of the aqueous layer), and final purification of the materials in question by preparative tlc on silica yielded 7.9 mg of compound **1** and 7.3 mg of compound **2**.

The eims of **1** and **2** both exhibited apparent molecular ions at *m/z* 521 and contained very prominent ions at *m/z* 267 and 128. Fragment ions at 221, 207, 192, 180, 167, and 154, characteristic of amide derivatives of lysergic acid (15), were also prominent in the spectrum. Positive ion cims yielded further evidence that 521 was the correct mol wt for **1** and **2** ($[MH]^+$ 522): both compounds gave strong $[AH]^+$ ions at *m/z* 268 typical of lysergic acid derivatives (13), and base peaks at *m/z* 255 characteristic of a typical $[BH]^+$ ion of ergot-peptide alkaloids (13) were observed. However, none of the common ergopeptine alkaloids are known to yield a major fragment ion at *m/z* 255. Negative ion cims of both **1** and **2** gave ions at 521 (apparent $[M]^-$), and base peaks were obsrved at *m/z* 254. The latter ion is also characteristic of the peptide portion of a typical lysergic acid peptide derivative. No fragment ions derived from the lysergic acid portion of such molecules are normally seen in the negative ion mode (13). Additional information concerning the peptide portion of **1** and of **2** was contained in the ms/ms daughter spectra of the $[B]^-$ ion (*m/z* 254). These spectra yielded ions *m/z* 211 and 183; once again, however, the ions did not match any that are characteristic of the known ergopeptine alkaloids.



1



2

Confirmation that **1** and **2** were indeed peptide derivatives of lysergic acid was obtained by comparison of their ^{1}H-nmr spectra with published spectra of ergotamine and its C-8 epimer (ergotaminine) (16) (Table 1). Inspection of Table 1 reveals that the ^{1}H spectrum of **1** had all of the signals expected for a derivative of lysergic acid and that their chemical shifts and coupling constants were nearly identical to those of the corresponding signals of ergotamine. By contrast, chemical shifts and coupling constants for protons assigned to the same positions of compound **2** corresponded much more closely to those of ergotaminine. Thus, it became apparent that **2** was the C-8 epimer of **1**. The ^{13}C assignments for **1** and **2**, when compared to assignments for ergocristine (17) and α-ergokryptine (18) (Table 2), lent additional support to these conclusions. Furthermore, purified **1** or **2** readily reverted to equilibrium mixtures of both epimers upon standing in solutions containing MeOH.

Having established that **1** and **2** were derivatives of lysergic acid and isolysergic acid, respectively, attention was focused on structure of the peptide unit. The ^{1}H signals at δ 1.52 in **1** and at δ 1.50 in **2** (Table 1) were indicative of a methyl group at the 2′ position as in ergotamine and ergotaminine (the 2′ position of typical ergot peptides). Also apparent was a typical 5′ proton signal (δ 4.52 in **1** and 4.42 in **2**) coupled to the methylene proton (δ 1.94) of an isobutyl group (as in α-ergokryptine). These conclusions were fully supported by signals in the ^{13}C spectra of **1** and **2** (Table 2) that were assigned to 2′, 3′, 5′, and to four carbons of an isobutyl group. Notably absent in both the ^{1}H and ^{13}C spectra were resonances that could be assigned to the 8′, 9′, and 10′ methylenes of a proline residue. However, the spectra of **1** and **2** exhibited methine

TABLE 1. ^{1}H-nmr Assignments for Ergobalansine [**1**], Ergobalansinine [**2**], Ergotamine, and Ergotaminine.[a]

Proton	Compound			
	1	**2**	Ergotamine[b]	Ergotaminine[b]
H-1	7.99 s	8.05 s	8.14	8.00
H-2	6.93 m	6.90 m	6.91	6.91
H-4a	2.82 m (14.3, 12.2)	3.59 m (14.4, 5.3)	2.79 (14.2, 11.9)	3.59 (15.3, 4.9)
H-4b	3.32 m (14.3, 5.0)	2.64 m (14.4, 12.0)	3.32 (14.2, 5.0)	2.62 (15.3, 10.4)
H-5	3.79 m (12.2, 5.0)	3.20 m (12.0, 5.3)	3.73 (11.9, 5.0)	3.23 (10.4, 4.9)
6-NMe	2.64 s	2.61 s	2.61	2.61
H-7a	2.98 dd (12.0, 3.7)	3.12 m (11.9)	2.96 (11.9, 3.9)	3.13 (11.7, 1.7)
H-7b	2.88 dd (12.0, 2.8)	2.75 (11.9, 3.5)	2.78 (11.9, 3.4)	2.76 (11.7, 3.8)
H-8a	3.18 m (5.6, 3.7, 2.8)	—	3.18 (5.5, 3.9, 3.4)	—
H-8b	—	3.06 m (6.3)	—	3.07 (6.3)
H-9	6.35 dd (5.6)	6.49 dd (6.3)	6.34 (5.5)	6.52 (6.3)
8-NH	9.26 s	9.85 s	9.04	9.83
H-12–14	7.18 m	7.15 m	7–7.5	—
2′-Me	1.52 s	1.50 s	1.51	1.49
H-5′	4.52 dd	4.42 dd	4.69	4.61
5′-CH_2	≅1.94 m	≅1.90 m	—	—
5′-CH_2CH	≅1.94 m	≅1.90 m	—	—
5′-$CH_2CH(CH_3)_2$	1.05 d	0.96 d	—	—
	1.00 d	0.95 d	—	—
H-8′–10′	not obs.	not obs.	obs.	obs.
H-11′	3.56 m	3.51	—	—
11′-CH_3	1.34 d	1.32 d	not obs.	not obs.
OH	5.84 s	6.01 s	6.97	6.94
7′-NH	6.78 bs	6.77 br s	not obs.	not obs.

[a]Nmr spectra were obtained with a Bruker WM-300 instrument in $CDCl_3$ solutions with TMS as an internal standard. Chemical shifts (δ) are expressed in ppm from TMS, and coupling constants *J*, in parentheses, are expressed in Hz. Assignments were confirmed utilizing COSY proton shift correlation experiments.

[b]Assignments were obtained from Pierri *et al.* (16) for comparison.

proton signals at δ 3.56 and 3.51, respectively, that were each coupled to both a methyl group and to an exchangeable (NH) proton. Both of the latter groupings, a methyl coupled to an apparent C-11′ proton and an exchangeable proton also coupled to the C-11′ proton, are not present in spectra of the other known ergot-peptide alkaloids. However, the ^{13}C spectra of **1** and **2** did have signals that could be assigned to C-11′ and C-12′. [It should be noted that the numbering system that we have used for the peptide portions of **1** and **2** is for convenience in comparing their nmr spectra with the usual ergopeptine alkaloids; thus, 11′ and 12′ should actually be 8′ and 9′, respectively.]

Structures of **1** and **2** assigned tentatively on the basis of their nmr spectra were confirmed by further consideration of their mass spectral fragmentation patterns. The scheme outlined (Figure 2) summarizes our interpretation of major fragment ions observed in cims and is consistent with nmr structural information and with analogous $[AH]^+$, $[BH]^+$, and $[CH]^+$ ions observed in previous studies of ergopeptines (13). In the negative ion daughter mode, **1** gave a base peak at *m/z* 183 $[C-H]^-$, as expected. The $[C]^+$ ion (*m/z* 184) is prominent in the eims, and the major ion at *m/z* 128 is readily explained by loss of isobutane from $[C]^+$. This latter ion corresponds to the *m/z* 154, which is diagnostic for ergopeptines that contain a proline residue (19). Thus, except for stereochemistry, the structures of **1** and **2** must be as shown.

The ergopeptines consist of a D-lysergic acid moiety linked to a tricyclic peptide moiety (6), and the 6 asymmetric centers are indicated by asterisks (Figure 1). We have assumed that **1** is also derived from D-lysergic acid; thus, the relative (and absolute) configurations at the 5 and 8 positions of **1** and **2** should be correct as shown. Configurations at the remaining asymmetric centers, as shown, are the same as for the normal series of ergopeptines obtained from *Claviceps* species; however, configurations at the 2′, 5′, 11′, and 12′ positions of **1** and **2** remain to be determined.

TABLE 2. ^{13}C-nmr Assignments for Ergobalansine [**1**], Ergobalansinine [**2**], Ergocristine, and α-Ergokryptine.[a]

Carbon	Compound			
	1	**2**	Ergocristine[b]	α-Ergokryptine[b]
C-2	119.0	118.4	119.2	(119.2)
C-3	110.8	110.1	110.6	110.6
C-4	22.1	27.7	30.9	26.5
C-5	59.6	62.6	59.3	64.5
6-NMe	41.1	43.6	40.9	44.3
C-7	48.9	54.5	48.2	48.1
C-8	44.1	43.0	44.3	40.9
C-9	118.4	117.6	118.8	(118.8)
C-10	139.2	137.5	138.9	139.2
C-11	129.5	127.5	129.6	129.6
C-12	112.0	112.8	111.9	111.9
C-13	123.4	123.3	123.3	123.3
C-14	110.0	110.2	110.2	110.1
C-15	133.8	133.9	133.8	113.9
C-16	126.2	126.2	125.9	126.3
17-CONH	175.7	176.0	176.2	176.2
C-2′	85.4	85.4	89.9	89.7
C-3′	(166.3)	(165.7)	(165.7)	(165.8)
C-5′	53.2	53.0	56.8	53.3
C-6′	(169.1)	(169.1)	(165.4)	(166.2)
C-8′	not obs.	not obs.	46.1	46.0
C-9′	not obs.	not obs.	21.7	21.6
C-10′	not obs.	not obs.	22.4	22.2
C-11′	57.0	56.9	64.3	59.3
C-12′	102.7	102.5	103.7	103.4
2′-Me	24.6	24.5	—	—
5′-CH_2	43.5	43.4	—	43.5
5′-CH_2CH	24.7	24.6	—	25.1
5′-$(Me)_2$	21.9, 22.8	22.0, 22.7	—	22.2, 22.6
11′-CH_3	14.7	14.7	not obs.	not obs.

[a]Nmr spectra were obtained with a Bruker WM-300 instrument in $CDCl_3$ solutions with TMS as an internal standard, and multiplicities were confirmed in DEPT experiments. Chemical shifts (δ) are expressed in ppm from TMS. Assignments were confirmed by COSY and 2D heteronuclear correlation experiments. Values in parentheses may be interchanged.

[b]Assignments were obtained from Stuchlik *et al.* (17) and Flieger *et al.* (18) for comparison.

A great deal of literature concerning ergopeptine alkaloids has accumulated over the years due to their wide range of biological activities and the extreme potency of certain derivatives (6,20). Ergotamine, ergonovine, and a few other naturally occurring or semisynthetic ergot alkaloids are routinely used in medical practice.

Biosynthesis of these compounds has also attracted considerable attention (21,22). It is generally accepted that attachment of L-proline to an enzyme is crucial as a starting point in biosynthesis of the peptide unit. This step is thought to be followed by relatively nonspecific attachment of two additional amino acid units before the resulting tripeptide unit is linked to lysergic acid. Proline has been considered to play a key role in ergopeptide biosynthesis and, except for a report that 1-thiazolidine-4-carboxylic acid will serve as a substitute (23), a proline residue has always been observed in the naturally occurring ergopeptines. Which specific amino acids are subsequently incorporated into the remainder of the peptide unit is at least partially controlled by the relative amino acid concentrations in the internal pool of the cells. *B. obtecta* and *B. cyperi*

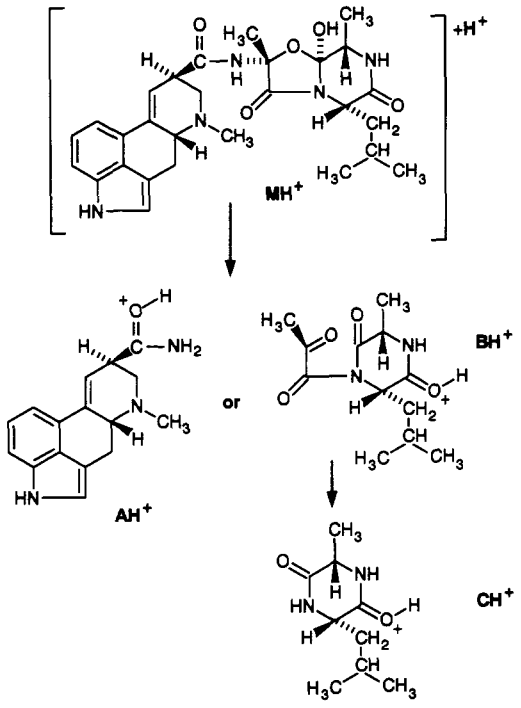


FIGURE 2. Positive ion cims fragmentation scheme for ergobalansine [**1**].

appear to be less specific in their requirements for ergopeptine biosynthesis, and as evidenced by compounds **1** and **2**, alanine serves as a starter unit for ergot-peptide biosynthesis in these species.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Analytical and preparative tlc was carried out on Si gel 60 F-254 plates (E. Merck) developed with $CHCl_3$-MeOH (9:1). Si gel (60–200 mesh, Baker) was used for cc. Preparative hplc utilized a Spectra-Physics SP8700XR solvent delivery system, a Gilson 231 automatic injector, a Rainin Dynamax-60A (21.4 mm × 250 mm) silica column with isocratic elution using CH_2Cl_2-MeOH (93:7), a Hitachi UV detector, and a Gilson 201 fraction collector. Analytical programmed-gradient hplc utilized a Spectra-Physics SP8800 ternary pump, a Varian Fluorichrom fluorescence detector (excitation at 310 nm, 375–460 nm light emission filter), a DuPont Zorbax column (4.6 × 250 mm, 5 mm ODS), and was conducted at a solvent flow rate of 0.8 ml/min. Solvents were MeCN and 0.1 N ammonium acetate buffer (pH 7.6). The previously used solvent program (12) was modified to the following: begin program at 35% MeCN and change linearly to 50% MeCN (55 min); hold at 50% MeCN (5 min); linearly to 100% MeCN (15 min); hold at 100% MeCN (20 min); then re-equilibrate column at 35% MeCN (30 min). Semiquantitative estimates of alkaloid concentrations were obtained by the peak height method. ^{1}H- (300 MHz) and ^{13}C- (75.47 MHz) nmr spectra were recorded with a Bruker WM 300 spectrometer using $CDCl_3$ as the solvent and TMS as an internal standard. Mass spectra were obtained with a Finnigan MAT 4535/TSQ instrument equipped with a DEP probe. Ms and tandem ms techniques specific for determination of ergopeptine alkaloids in complex matrices were conducted as described previously (13).

PLANT AND FUNGAL MATERIALS.—Collections of *Ce. echinatus* infected with *B. obtecta* were made and authenticated by Keith Clay November 24, 1987, along roadsides near Frostproof and Haines City, Polk County, Florida. *C. virens* infected with *B. cyperi* was collected from a marsh near Cameron Parish, Louisiana, June 1, 1987. Voucher specimens have been deposited in the National Mycological Herbarium in Beltsville, Maryland. Cultures of *B. obtecta* and *B. cyperi* were isolated from the respective plant materials and maintained on corn meal agar slants.

EXTRACTION AND FRACTIONATION OF *CE. ECHINATUS*.—Aerial parts of *Ce. echinatus* infected with *B. obtecta* (9.0 kg) were ground in a Wiley mill fitted with a ¼-in screen, placed in a stainless steel

tank along with 20 gallons of 95% EtOH, and allowed to stand for 24 h. The EtOH was then drained from the plant material, and the extraction process was repeated three additional times. Combined EtOH extracts were evaporated to a volume of 6 liters, below 42°, on a rotary evaporator. Evaporation of an aliquot to dryness revealed that the EtOH concentrate contained 348 g of nonvolatile extractables.

A portion of the concentrate, containing approximately 50 g of the nonvolatile extractables, was placed in a round-bottomed flask along with 30 g of silica, and the excess EtOH was removed on a rotary evaporator under reduced pressure. A 6 cm × 70 cm glass column was prepared that contained CH_2Cl_2 and 350 g of silica. The silica that had been coated evenly with 50 g of extract was applied to the top of the column, and the column was eluted with the following solvent series: CH_2Cl_2 (1.5 liters), 5% MeOH in CH_2Cl_2 (1.5 liters), 10% MeOH in CH_2Cl_2 (1.5 liters), and 25% MeOH in CH_2Cl_2 (1.5 liters). Collected fractions (200 ml each) were analyzed by tlc and recombined into 8 fractions on the basis of similarity of tlc patterns observed. The remaining extract was processed by dividing it into 6 portions and repeating the entire chromatographic procedure 6 additional times. Like materials were combined, and all 8 fractions were examined for the presence of ergot-peptide alkaloids using published ms (13) and hplc techniques (14). Fractions 5–7, those materials having R_f's of 0.5–0.9 (10% MeOH in $CHCl_3$), contained compounds that were derivatives of lysergic acid; however, none of the known ergot-peptide alkaloids were detected. Thus, fractions 5 (14.3 g), 6 (5.0 g), and 7 (7.5 g) were selected for further study.

An alkaloid-enriched fraction was prepared from column fraction 5 by solvent partitioning in the following manner: The entire sample, 14.3 g, was dissolved in 400 ml of EtOAc and 800 ml of aqueous 2% tartaric acid in a 2-liter separatory funnel. The layers were mixed and allowed to separate, and the aqueous layer was partitioned a second time with 400 ml of EtOAc. The aqueous layer was then made basic, to pH 9, by slow addition of concentrated NH_4OH. The resulting basic solution was then extracted 3 times with 400-ml portions of EtOAc, and the combined EtOAc extracts yielded 204 mg of alkaloidal material. Column fractions 6 and 7 were treated in a similar fashion, and these yielded 140 mg and 586 mg of alkaloidal materials, respectively. Analysis of the alkaloid-enriched fractions by reversed-phase hplc revealed that all three were enriched in two highly fluorescent compounds. The first of these, compound **1**, eluted slightly before ergovaline, and the second, compound **2**, eluted between ergotamine and ergovalinine. The alkaloid concentrates were further enriched in compounds **1** and **2** by repeated hplc on silica, and final purification of both was achieved by preparative tlc. Purified compound **1** or **2** readily reverted (epimerized) to an approximate 1:1 equilibrium mixture of **1** and **2** on standing in solutions containing MeOH, particularly at elevated temperatures.

ERGOBALANSINE [**1**].—Compound **1**, 7.9 mg, was obtained as a white solid in 0.00008% yield (based on dried *Ce. echinatus* plant material): ^{1}H nmr see Table 1; ^{13}C nmr see Table 2; eims *m/z* (rel. int.) $[M]^+$ 521 (3), 337 (6), 267 (100), 224 (30), 221 (52), 207 (49), 196 (21), 184 (38), 180 (29), 167 (19), 154 (22), 141 (17), 128 (54), 113 (21), 44 (48), 43 (59); cims (isobutane) *m/z* (rel. int.) $[MH]^+$ 522 (6), 504 (2), 268 (93), 255 (100), 185 (12); negative ion cims (isobutane) *m/z* (rel. int.) $[M]^-$ 521 (36), 254 (100). The ms-ms daughter spectrum of *m/z* 254 (negative ion cims) was as follows: *m/z* 254 (27), 211 (7), 183 (100), 126 (2), 113 (2), 86 (4). Found for $[AH]^+$ (Figure 2) *m/z* 268.1422 ($C_{16}H_{18}N_3O$ requires 268.1450); $[BH]^+$ *m/z* 255.1362 ($C_{12}H_{19}N_2O_4$ requires 255.1345).

ERGOBALANSININE [**2**].—Compound **2** (7.3 mg) was obtained as a white solid in 0.00008% yield (based on dried *Ce. echinatus* plant material): ^{1}H nmr see Table 1; ^{13}C nmr see Table 2; eims *m/z* (rel. int.) $[M]^+$ 521 (3), 337 (4), 267 (64), 221 (43), 207 (47), 196 (25), 184 (36), 180 (34), 167 (22), 154 (33), 141 (25), 128 (96), 113 (33), 44 (89), 43 (100); cims (isobutane) *m/z* (rel. int.) $[MH]^+$ 522 (8), 504 (2), 268 (97), 255 (100), 185 (13); negative ion cims (isobutane) *m/z* (rel. int.) $[M-42]^-$, 254 (100). Found for $[AH]^+$ *m/z* 268.1412 ($C_{16}H_{18}N_3O$ requires 268.1450); $[BH]^+$ *m/z* 255.1362 ($C_{12}H_{19}N_2O_4$ requires 255.1345).

CULTURE OF *B. OBTECTA* AND *B. CYPERI* AND ANALYSIS OF EXTRACTS.—Alkaloid production was achieved using pure cultures and a two-stage fermentation procedure as described by Bacon *et al.* (24). Isolates were grown in a liquid sporulation medium and then transferred to a second medium with high sorbitol content, in which alkaloid biosynthesis occurred. Cultures were then incubated on a shaker table for 10 days and then allowed to remain stationary for 3–4 weeks at constant 24°. Mycelia were recovered by filtration.

A 2.1-g portion of freeze-dried mycelium of *B. obtecta* was moistened with 5 ml of H_2O and then extracted three times with 10-ml portions of CH_2Cl_2-MeOH (9:1). The CH_2Cl_2/MeOH extracts were combined and evaporated to dryness, yielding 5.7 mg of extractable material. Analysis of the extract by hplc revealed that the retention times of the two most prominent fluorescent peaks were identical to those of compounds **1** and **2**. A positive ion cims of the extract yielded prominent ions characteristic of both compounds **1** and **2** ($[MH]^+$ 522, 268, 255); negative ion cims of the extract also revealed ions at 521 $[M]^-$ and 254 characteristic of both **1** and **2**. Further confirmation of the presence of **1** and/or **2** was obtained by an ms-ms daughter spectrum of *m/z* 254 in the negative ion cims mode (10). Observed ions were at *m/z* (rel. int.) 254

(100), 211 (8), 183 (80), 126 (1), 113 (2), and 86 (3). Attempted quantitation by the peak height method indicated that the amounts of **1** and **2** present in the mixture totaled less than 5%.

In like manner, a 0.9-g portion of freeze-dried mycelium obtained from a culture of *B. cyperi* was moistened with 5 ml of H_2O and then extracted three times with 10-ml portions of CH_2Cl_2-MeOH (9:1). The CH_2Cl_2/MeOH extracts were combined and evaporated to dryness, yielding 2.7 mg of extractable material. Analysis of the extract by hplc revealed that the retention times of the two most prominent fluorescent peaks were identical to those of compounds **1** and **2**. Further confirmation of the presence of **1** and/or **2** was obtained by ms and ms-ms techniques as described above. Attempted quantitation revealed that the amounts of **1** and **2** present in the mixture totaled less than 2%. Analysis of a *B. cyperi* culture filtrate extract prepared as described by Plowman *et al.* (25) also revealed the presence of **1** and **2**; however, two of the most predominant alkaloids produced by *B. cyperi* were identified by ms, nmr, and comparison with known standards as chanoclavine I and festuclavine.

EXTRACTION AND FRACTIONATION OF *C. VIRENS*.—Aerial parts of *C. virens* observed to be infected with *B. cyperi* (5 kg) were ground in a Wiley mill and extracted repeatedly with 95% EtOH. Combined EtOH extracts were reduced to 1.6 liters, and evaporation of an aliquot to dryness revealed that the EtOH concentrate contained 192 g of nonvolatile extractables. The concentrate was chromatographed on silica, exactly as described for the *Ce. echinatus* extract, and similar materials were combined into 6 fractions based on tlc. The six fractions were examined by hplc, and evidence of trace amounts of compounds **1** and **2** was found in fractions 2 and 3. Fractionation of the extract was discontinued at this point as it was estimated that fractions 2 and 3 (19 g total) contained less than 1 mg of compounds **1** and **2**.

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