which supports $R_1 = C_3H_7$ and $R_2 = C_4H_9$. This analogy may be applied to the remainder of the CI mass spectra for these alkaloids.

Since no CI study was made on the epimeric species of these compounds, it is unknown if the observed differences among the α- and β-ergosine, ergoptine, and ergokrystine (Table I) may be used to differentiate the alkyl moieties when R_2 = isobutyl and/or *sec*-butyl. Also, it is unknown at present if the differences in ion intensities for C′ (Table I) is a reflection of the stability and/or ease of formation of C relative to the substituents R_1 and R_2. However, the data indicate that CI mass spectrometry is an effective and useful complement to the EI spectra for the identification of the ergot peptide alkaloids.

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LITERATURE CITED

Arsenault, G. P. In "Biomedical Application of Mass Spectrometry"; Waller, G. R., Ed.; Wiley-Interscience: New York, 1972.

Bacon, C. W.; Porter, J. K.; Robbins, J. D.; Luttrell, E. S. *Appl. Environ. Microbiol.* **1977**, *34*, 576.

Barber, M.; Weisbach, J. A.; Douglas, B.; Dudek, G. O. *Chem. Ind. (London)* **1965**, 1072.

Belzecki, C. M.; Quigley, F. R.; Floss, H. G.; Crespi-Perellino, N.; Guicciardi, A. *J. Org. Chem.* **1980**, *45*, 2215.

Berde, B.; Schild, H. O. "Ergot Alkaloids and Related Compounds"; Springer-Verlag: Berlin, Heidelberg, and New York, 1978; p 34.

Bové, F. J. "The Story of Ergot"; S. Karger: Basel, Switzerland, 1970; p 42.

Brunner, R.; Stütz, P. L.; Tscherter, H.; Stadler, P. A. *Can. J. Chem.* **1979**, *57*, 1638.

Burfening, P. J. *J. Am. Vet. Med. Assoc.* **1973**, *163*, 1288.

Fales, H. M.; Lloyd, H. A.; Milne, G. W. A. *J. Am. Chem. Soc.* **1970**, *92*, 1590.

Floss, H. G. *Tetrahedron* **1976**, *32*, 873.

Inoue, T.; Nakahara, Y.; Niwaguchi, T. *Chem. Pharm. Bull.* **1972**, *20*, 409.

Kohlmeyer, J.; Kohlmeyer, E. *Mycologia* **1974**, *66*, 77.

Porter, J. K.; Bacon, C. W.; Robbins, J. D. *J. Agric. Food Chem.* **1979**, *27*, 595, and references cited therein.

Schmidt, J.; Kraft, R.; Voight, D. *Biomed. Mass Spectrom.* **1978**, *5*, 674.

Sprague, R. "Diseases of Cereals and Grasses in North America (Fungi except Smut and Rusts)"; The Ronald Press Co.: New York, 1950.

Stadler, P. A., Sandoz Ltd., Basel, Switzerland, personal communication, 1979.

Stadler, P. A.; Stütz, P.; Stürmer, E. *Experientia* **1977**, *33*, 1552.

Voight, D.; Johne, S.; Groger, D. *Pharmazie* **1974**, *29*, 697.

Vokoun, J.; Rehacek, Z. *Collect. Czech. Chem. Commun.* **1975**, *40*, 1731.

Vokoun, J.; Sajdl, P.; Rehacek, Z. *Zentralbl. Bakteriol., Parasitenkd., Infektionskr. Hyg., Abt. 2, Naturwiss.: Allg., Landwirtsch. Tech. Mikrobiol.* **1974**, *129*, 499.

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Ergot Alkaloid Identification in Clavicipitaceae Systemic Fungi of Pasture Grasses

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The isolation and identification of an alkaloid from *Balansia epichloë*, *Balansia strangulans*, and *Epichloë typhina* that corresponds to 6,7-secoagroclavine (UV; TLC; *m/e*) are reported. We report on the production of agroclavine, elymoclavine, penniclavine, and festuclavine by *E. typhina*. In addition, the two ergot peptide alkaloids from *E. typhina* previously listed as ergosine and ergosinine when analyzed with isobutane chemical ionization mass spectroscopy corresponded to ergovaline and ergovalinine. Another systemic fungus, *Balansia henningsiana*, was shown to produce chanoclavine(s), dihydroelymoclavine, and another presently unidentified ergoline alkaloid.

Previous investigations (Bacon et al., 1979; Porter et al., 1978, 1979a,b) of systemic fungi from toxic pasture grasses established that *Balansia epichloë*, *Balansia claviceps*, *Balansia henningsiana*, *Balansia strangulans*, and *Epichloë typhina* produced clavine-type alkaloids in vitro. Bacon et al. (1979) showed that several alkaloids produced by *B. epichloë* in vitro were also produced in vivo on parasitized smut grass (*Sporobolus poiretii*). These studies suggest that these systemic grass pathogens should be suspect in ergot toxicity syndromes of cattle and that "ergot" alkaloid biosynthesis occurs in other genera of Clavicipitaceae. The toxins responsible for the "ergot-like" syndromes observed in cattle have not been established. Therefore, it is important to characterize the alkaloids produced by these systemic grass pathogens.

Laboratory studies have shown similarities in the capability of *Balansia* and *Epichloë* to produce clavine al-

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kaloids. The major differences in alkaloid biosynthesis between *Balansia* and *Epichloë* is that *E. typhina* may be distinguished by its ability to produce ergot peptide alkaloids similar to *Claviceps purpurea* (Brunner et al., 1979). In addition to the alkaloids that chromatographed with ergosine and ergosinine (Porter et al., 1979a) and chanoclavine I, we also report the identification of agroclavine, elymoclavine, penniclavine, and 6,7-secoagroclavine from a 20-week culture of *E. typhina*. All of these clavine alkaloids correspond to authentic standards as described (Fehr, 1967; Porter et al., 1978, 1979b). The two ergot peptide fractions labeled ergosinine and ergosine (Porter et al., 1979a) also corresponded with synthetic standards except for the minor discrepancies (i.e., ion intensities) in the low-resolution mass spectra as reported. These, along with an absence of the molecular ion, the low abundance of the diagnostic fragments of *m/e* 280, 210, and 86 amu, and the close similarity of the spectra of the other homologous alkaloids (Brunner et al., 1979; Stadler et al., 1977), prompted further investigations into the identity of the ergot peptide alkaloids produced by *E. typhina*. Comparative TLC and low-resolution mass spectra eliminated all of the natural and synthetic alkaloids of the ergotoxine and ergoxine groups (Brunner et al., 1979) and all but ergosine and ergovaline from the ergotamine group. Interpretation of ergovaline's mass spectra (i.e., electron impact, EI, at 70 eV) is complicated by the fragmentation of the tricyclic peptide moiety resulting in the same atomic mass ions as the lysergic acid amide and clavine portions (Vokoun et al., 1974; Vokoun and Rehacek, 1975) of the alkaloid. The above is further compounded by the almost identical chemical and physical properties of ergosine and ergovaline (Brunner et al., 1979) and the problems of working with small quantities of a natural isolate (Porter et al., 1979a). Chemical ionization (CI) mass spectroscopy's (Arsenault, 1972; Fales et al., 1970) compliment to the low-resolution electron impact (EI) has been reported for differentiation of the alkyl substituents attached to the tricyclic peptide moiety of these alkaloids (Porter and Betwoski, 1981). Therefore, CI mass spectroscopy was used to compare the natural peptide alkaloids from *E. typhina* with authentic ergosine and ergovaline.

Cultures of *B. epichloë* and *B. strangulans* were reported (Bacon et al., 1979; Porter et al., 1979a,b) to produce a clavine alkaloid (M^+ 240) that we now report as 6,7-secoagrocalvine. The identification was based on the comparison with a synthetic standard using ultraviolet (UV) and mass spectroscopy, cochromatography, and color reaction (blue) with *p*-(dimethylamino)benzaldehyde (PDAB) and also by a comparison of the methylated derivative of the natural alkaloids with synthetic *N*-methyl-6,7-secoagroclavine (Fehr, 1967). The 6,7-secoagroclavine has been recently reported as a natural product from *C. purpurea* (Horwell and Verge, 1979).

EXPERIMENTAL SECTION

Organisms, culture, and alkaloid extraction, isolation, and detection methods were as previously described: *B. epichloë* (RRC 242), alkaloid no. 1 (Porter et al., 1978, 1979b); *B. strangulans* (RRC 233), alkaloid fraction (Bacon et al., 1979) and alkaloid *m/e* 240 (Porter et al., 1978); *E. typhina* (RRC 238), ergot peptide alkaloids labeled ergosine and ergosinine (Porter et al., 1979a); *E. typhina* (RRC 238), a 20-week culture on media 104 (Porter et al., 1979a). In addition, *E. typhina* (RRC 238) was cultured for 12 weeks on 100 g of sorbitol, 10 g of glutamic acid, 1.0 g of yeast extract, 1.0 g of KH_2PO_4, 0.3 g of $MgSO_4 \cdot 7H_2O$, and 1000 mL of distilled water, and the pH adjusted to 5.6 with NH_4OH, and the *B. henningsiana* (RRC 243) alkaloid fraction obtained from Bacon et al. (1979).

Chromatography. Thin-layer chromatography was performed on silica gel GF-254 according to reported procedures (Agurell, 1965; Porter et al., 1979a; Stahl, 1969) with the following solvent systems: chloroform–methanol (CM), 4:1 (v/v); chloroform–dimethylamine (CDEA), 9:1 (v/v) (Agurell, 1965); benzene–dimethylformamide (BDMF), 86.5:13.5 (v/v) (Stahl, 1969); chloroform–methanol–concentrated ammonia (CMA), 94:5:1 (Horwell and Verge, 1979); chloroform–methanol, 9:1 (v/v) in a saturated ammonia atmosphere (CMAtm) (Cassady et al., 1973); methylene chloride–2-propanol (MP), 3:1 (v/v).

Instrumental. Low-resolution electron impact mass spectra were obtained as reported (Porter et al., 1979a,b). Chemical ionization mass spectra were obtained via a direct inlet probe on a Varian Mat 44 quadrapole mass spectrometer equipped with an EI/CI source (~200 V). The ion source was maintained at a pressure of ~200 μbar and operated at a temperature of ~245 °C. The probe temperature was programmed at 20 °C/min from ambient to 250 °C. The fragment ions were observed between 175–200 °C. Only those ions occurring at a relative intensity greater than 1% are recorded. Isobutane was used as the reactant gas, and (perfluorotributyl)amine (*m/e* 219, 264, and 414 amu) was used as a calibration compound. Ultraviolet spectra were performed in methanol by using a Varian Cary 15 ultraviolet spectrometer. Infrared spectra were performed using KBr micropellets in a Perkin-Elmer Model 457A infrared spectrometer. Nuclear magnetic resonance spectra were performed in CD_3OD by using a JEOL PS/PFT 100. Melting points were uncorrected and obtained with a Mettler FP5 apparatus connected to a Mettler FP52 microfurnace.

Synthesis of 6,7-Secoagroclavine. *N*-Methyl-6,7-secoagroclavine [M^+ 254; mp 132–134 °C [lit. mp 136 °C (Fehr, 1967)]] was synthesized from agroclavine (Eli-Lilly) according to Bhattacharji et al. (1962) and separated from its Δ^7 isomer [M^+ 254; mp 120 °C [lit. mp 118–120 °C (Fehr, 1967)] by preparative TLC on silica gel GF 254 by using the CM solvent system (R_f 0.57 and 0.50, respectively). The *N*-methyl-6,7-secoagroclavine (0.4 mM) in 2 mL of anhydrous acetone was treated with 0.7 mM of diethyl azodicarboxylate (Aldrich Chemical Co.) in 3 mL of anhydrous diethyl ether. The reaction mixture was stirred under N_2 for 5 h and allowed to stand in the refrigerator overnight. The reaction mixture was extracted (3 × 3 mL) with a 2% tartaric acid solution. Extracts were combined, the pH was adjusted to ~10 with NH_4OH, and the resulting solution was extracted with 3 times the equivalent volume of $CHCl_3$. The alkaloidal extracts were combined, treated with anhydrous Na_2SO_4, filtered, and subjected to preparative TLC (20 × 20 cm glass plates, 0.75-mm silica gel GF-254) using the CDEA solvent system. The *N*-methyl-6,7-secoagroclavine (R_f 0.70; M^+ 254) starting material separated from the reaction products (R_f 0.60) in this system. Low-resolution mass analyses of the reaction product(s) isolated from the silica gel suggested it consisted of at least three compounds: M^+ 252, *m/e* 237 (252 – CH_3); M^+ 240, *m/e* 225 (240 – CH_3); M^+ 176, *m/e* 131 (176 – OC_2H_5). The desired product (M^+ 240) was isolated from this mixture by preparative TLC using the BDFA solvent system (R_f 0.37). After elution from the silica gel, this fraction was chromatographed in the CM solvent system (R_f 0.23), followed by recrystallization from hexane [mp 132 °C [lit. mp 138 °C (Fehr, 1967); lit. mp 126–129 °C (Horwell and Verge, 1979)]], and the isolated compound had a UV, IR, and NMR as reported (Fehr,

1967). Also isolated in this chromatography system was a fraction (R_f 0.57) that was visually observed as a dark blue band under 254 and 366 nm and gave a blue reaction to PDAB. Its absorption ($\lambda_{max}^{CH_3OH}$ 317 and 236 nm) along with the low-resolution mass spectrum (M^+ 252, m/e 237, 221, 206, 196, 181, 167, 154, and 85) gave indications that this fraction consisted mainly of a dehydrogenated *N*-methyl-6,7-secoagroclavine with the newly formed double bond being conjugated with the indole nucleus (Cassady et al., 1973). The mass spectrum also revealed the presence of diethyl hydrazinodicarboxylate (Fieser and Fieser, 1967; Yoneda et al., 1966), M^+ 176, m/e 131 and 130 amu, as a minor constituent.

Methylation of Natural Products (M^+ 240) Isolated from *B. epichloë* and *B. strangulans*. Methanol, 200 μL, and 10 drops of methyl iodide (Aldrich Chemical Co.) were added to sufficient chromatography material (~250 μg) of each natural alkaloid. The systems were flushed with N_2, capped, and allowed to stand for 45 min at 40–45 °C with periodic manual agitation. The reaction mixtures were concentrated under a stream of N_2 to 100 μL, and each was subjected to preparative TLC in CDEA as above. This system separated starting material from the semisynthetic product (M^+ 254), indicative of the addition of one methyl substitutent.

Festuclavine from *E. typhina* (Sorbitol–Glutamic Acid Culture Media). The alkaloid fraction (Porter et al., 1979a) from a 12-week culture was chromatographed on silica gel by using CM and was visualized by spraying with PDAB. The one band corresponding to a blue reaction for ergot alkaloids was scrapped from the TLC plate, eluted from the silica with chloroform–methanol, 1:1 (v/v), and chromatographed with authentic pyro-, costa-, and festuclavine in solvent systems CDEA and BDMF. The natural alkaloid from this culture corresponded to festuclavine as determined by UV, TLC, and m/e (Agurell, 1965; Vokoun et al., 1974).

Clavine Alkaloids from *B. henningsiana*. The alkaloid fraction (625 μg, based on ergonovine maleate) isolated from a 5-week culture of *B. henningsiana* (Bacon et al., 1979) was subjected to preparative TLC in solvent system CM and resulted in three fractions (R_f 0.10, 0.18, and 0.65) that gave a blue reaction with PDAB. Although fraction 1 (R_f 0.10) corresponded to chanoclavine I in CDEA (Agurell, 1965) and CMAtm (Cassady et al., 1973; Porter et al., 1978) and its low-resolution mass spectrum showed M^+ 256, m/e 237, 183, and 154 as reported (Vokoun et al., 1974), the quantity of material isolated was not sufficient to unequivocally eliminate the isomeric relatives. Fraction 2 (R_f 0.18) cochromatographed with authentic dihydroelymoclavine (dihydrolysergol I, Eli Lilly) in CM (R_f 0.18), CDEA (R_f 0.15), CMAtm (R_f 0.47), and MP (R_f 0.04). Comparison of the low-resolution mass spectra of this fraction and dihydroelymoclavine (Table I) conducted under identical conditions showed discrepancies which may be the results of compound purity or an isomer that will not separate by chromatography under the conditions described. Attempts to compare the acetylation material of fraction 2 with *O*-acetyldihydroelymoclavine synthesized according to Agurell et al. (1963) were unsuccessful, probably because of the quantity of the compound originally isolated. Fraction 3 (CM, R_f 0.65) had a $\lambda_{max}^{CH_3OH}$ and low-resolution mass spectrum (Figure 1) suggestive of either an isomer of dihydrolyseramide [CM, R_f 0.26 [lit. R_f 0.23 (Agurell, 1965)]] or a derivative of this compound, i.e., RCONHR′ for R = ergoline [cf. m/e 144, Figure 1 (Barber et al., 1965; Schmidt et al., 1978)] with the R′ unknown. Fermentation conditions for *B. henningsiana* and the other systemic fungi mentioned above are under investigation in order to provide material sufficient for absolute identification of these alkaloids.

Table I. Mass Fragmentation Comparison of Dihydroelymoclavine and Alkaloid Fraction 2 from *B. henningsiana* (cf. the Text)

	%	
m/e	standard	natural product
257 (M + 1)	15	15
256 (M)	68	55
241	5	5
239	2	7
237	3	7
225	8	13
223	16	15
211	2	15
209	6	13
207	5	16
197	24	25
195	7	10
194	9	16
182	15	22
168	26	27
167	35	39
159	1	16
156	14	17
155	31	36
154	100	100
153	13	22
144	39	39
127	36	45
115	13	21

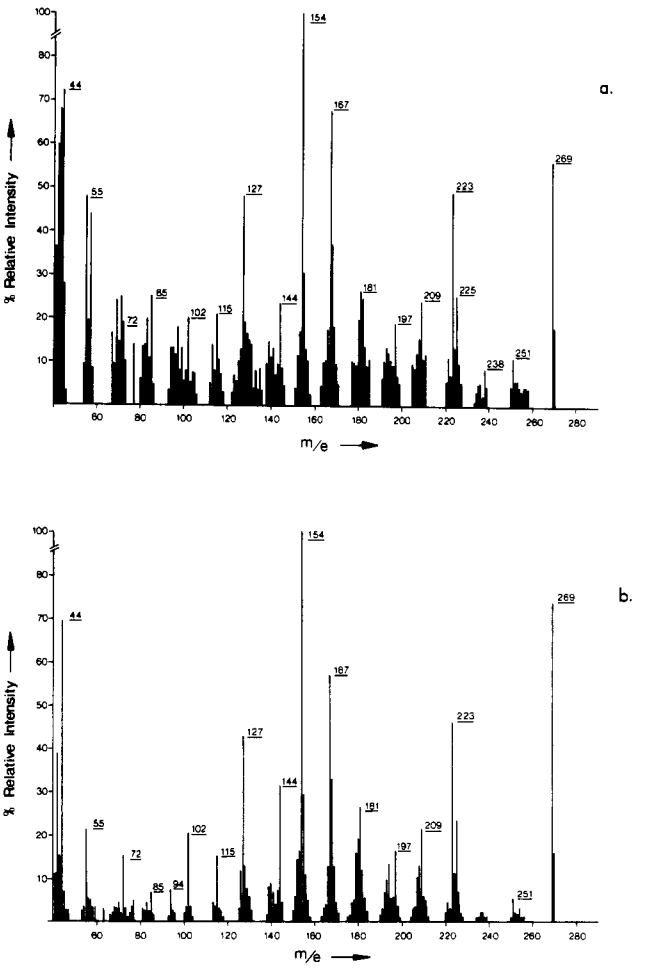

Figure 1. (a) Alkaloid (fraction 3) from *B. henningsiana* (cf. the text); (b) dihydrolyseramide.

Table II. Major Diagnostic Mass Fragments of Synthetic (syn.) 6,7-Secoagroclavine (Fehr, 1967) and Alkaloid(s) Isolated from Systemic Fungi *B. epichloë* (B.e.), *B. strangulans* (B.s.), and *E. typhina* (E.t.)

	%			
m/e	syn.	B.e.	B.s.	E.t.
240	100	100	100	100
225	10	12	10	17
208		12	12	17
197	16	15	18	37
194		20	21	27
184	22	25	28	32
182		23	26	46
168	37	42	45	54
155	59	58	79	82
127	10	18	26	37
115		18	20	15
85	56	75	79	85
42	17			29

RESULTS AND DISCUSSION

Alkaloid Workup of 20-Week Culture of *E. typhina* (RRC 238) on M 104. Preparative chromatography of the alkaloid fraction from Porter et al. (1979a) on silica gel GF 254 using CM resulted in six fractions that cochromatographed (R_f) with (1) ergosinine (0.74), (2) ergosine (0.60), (3) agroclavine (0.35), (4) penniclavine (0.26), (5) elymoclavine (0.21), and (6) chanoclavine(s) (0.09). After elution from the silica gel, these individual fractions chromatographed as homogeneous spots in the CM system with the authentic standards listed. Three of these fractions separated into additional compounds on chromatography in the CDEA solvent system. Fraction 1 separated into the alkaloid that cochromatographed with ergosinine and a minor compound that was visualized as a blue spot only after spraying with PDAB. This minor compound (R_f 0.76) was separated from ergosinine by developing the plates 2 times in CDEA and allowing the plates to air dry between runs. Although this minor component appeared homogeneous in CM (R_f 0.74), CDEA (R_f 0.54), and BDMF (R_f 0.79) and its UV spectrum suggested it was a simple indole or clavine alkaloid ($\lambda_{max}^{CH_3OH}$ 292, 281, 273, and 224 nm), the low-resolution mass analyses of this fraction suggested a complex mixture of several compounds. As the probe temperature of the mass spectrometer was increased in 50 °C increments from ambient to 250 °C, major ions were observed at 100 °C (*m/e* 244, 153, 125, 91, and 70 amu) and 250 °C (*m/e* 369, 314, 297, 270, 255, 237, 225, 207, 194, 192, 181, 168, 167, 154, 143, and 127 amu), respectively. The above spectra may represent two compounds, one of which may be (or contain) the phenylalanylproline lactam moiety (Groger et al., 1975); also, the observed spectra may represent one compound that is subject to pyrolyses prior to electron impact similar to the ergot cyclol alkaloids (Porter and Betowski, 1981). This fraction was stored under N_2 (0 °C) for future investigations.

When subjected to preparative chromatography in the CDEA system, fraction 5 separated into elymoclavine (Porter et al., 1979b) and another compound that corresponded [UV; TLC; *m/e* (Table II)] to the synthetic 6,7-secoagroclavine. Fraction 6 in the CDEA system consisted mainly of chanoclavine I (UV; TLC; *m/e*) and a few minor compounds that were not investigated.

The natural alkaloids [M^+ 240 (Table II)] from *B. epichloë* (Porter et al., 1978, 1979b), *B. strangulans* (Bacon et al., 1979; Porter et al., 1978), and *E. typhina* had identical chromatography behavior with synthetic 6,7-secoagroclavine in the CM (R_f 0.21), CDEA (R_f 0.57), and CMA systems with R_f values relative to those of elymoclavine and agroclavine (Horwell and Verge, 1979).

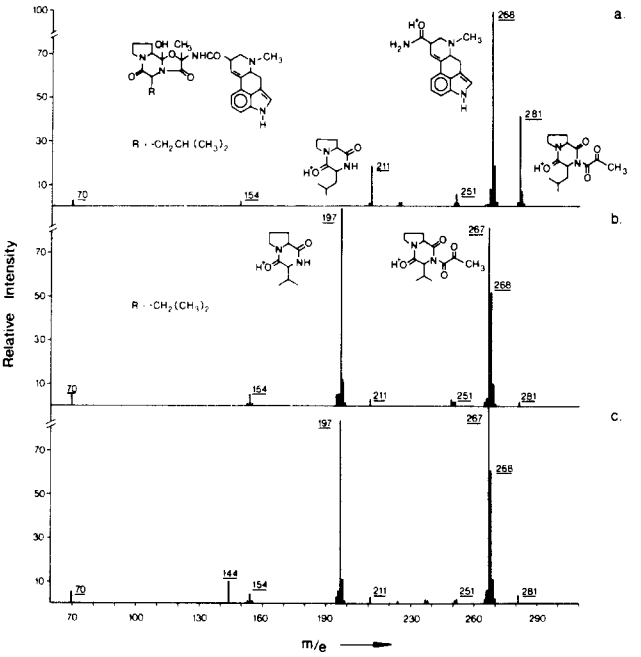

Figure 2. Isobutane chemical ionization mass spectra: (a) ergosine; (b) ergovaline; (c) peptide alkaloid (fraction 1) from *E. typhina* (cf. the text).

Although the natural product could not be separated from elymoclavine in CM and agroclavine in CDEA (Porter et al., 1979b), these two systems along with CMA (Horwell and Verge, 1979) may be used effectively for the preparative TLC isolation of this compound.

The low-resolution mass analyses for the above alkaloids are compared with that of 6,7-secoagroclavine (Fehr, 1967) in Table II. Also, the methylation product(s) of the above compounds corresponded with synthetic *N*-methyl-6,7-secoagroclavine on cochromatography in CM and CDEA as described (cf. synthesis).

The above data strongly suggest that the natural compounds isolated from *B. epichloë*, *B. strangulans*, and *E. typhina* are 6,7-secoagroclavine; however, paucity of the isolated materials prevented unequivocal establishment of conformation and therefore does not rule out the possibility of C-5, C-10(H) epimeric species.

Isobutane Chemical Ionization Mass Spectrometry of the Peptide Alkaloids from *E. typhina*. The electron impact (70 eV) mass spectrum of ergosine has been reported (Vokoun et al., 1974; Vokoun and Rehacek, 1975). The chemical ionization mass spectrum of ergosine is compared with that of ergovaline and the natural peptide alkaloid isolated from *E. typhina* in Figure 2. Under CI, the peptide fragment for ergosine corresponding to *m/e* 280 (Vokoun and Rehacek, 1975) undergoes the ion–molecule reaction with isobutane, thereby producing the abundant fragment at *m/e* 281 [42% (Figure 2a)]. Alternatively, ergosine may decompose via the diketopiperazine pathway (Porter and Betowski, 1981; Vokoun et al., 1974; Vokoun and Rehacke, 1975) which then undergoes the same reaction with isobutane, thus yielding *m/e* 211 (18%) (EI, *m/e* 210) and supporting R = C_4H_9. The lysergic acid amide fragment corresponding to *m/e* 267 undergoes the same mechanism, thereby resulting in *m/e* 268 (100%) for ergosine (Figure 2a). Similarly, the natural product from *E. typhina* (Figure 2c) resulted in *m/e* 267 (100%) and *m/e* 197 (95%), the peptide fragments indicative of R = C_3H_7 (Porter and Betowski, 1981). These two fragments corresponded to EI *m/e* 266 and 196, respectively (Porter et al., 1979a), and thus strongly support the identification of the natural compound from *E.*

typhina as ergovaline. The above ions for the natural product (Figure 2c) are also supported by the abundant lysergic acid amide fragment at m/e 268 (61%). The CI spectrum of natural ergovaline (i.e., from *C. purpurea*) is compared with the spectrum of the peptide alkaloid isolated from *E. typhina* (parts b and c of Figure 2, respectively). The minor differences observed in the relative intensities of m/e 267 and 197 between the two compounds may be a reflection of both instrument temperature and pressure at which pyrolysis and subsequent fragmentation occurs (Porter and Betowski, 1981) and possibly concentration of epimeric species. Figure 2b is the CI spectrum of ergovaline, whereas Figure 2c represents the spectrum of the epimeric mixture (i.e., ergovaline–ergovalinine) as determined by TLC.

The above natural standard (Figure 2b) was isolated from a fraction containing ergosine (Brunner et al., 1979), and thus it is unknown at present if the minor fragments occurring at m/e 281 and 211 in both spectra (Figure 2b,c) represents trace amounts of ergosine (Figure 2a). Pure synthetic ergovaline was unavailable for these comparisons. Thus, the possibility exists that *E. typhina* produces ergosine and ergosinine (Porter et al., 1979a) in trace amounts with ergovaline and ergovalinine as the major components.

The above comparisons do not eliminate the possibility of the peptide alkaloids from *E. typhina* as being isomeric relatives of ergovaline and ergovalinine. Conformational analyses of these alkaloids, as with the clavine alkaloid from *Balansia*, are predicated on quantities sufficient for these determinations.

Although the synthetic peptide alkaloids ergovaline and ergovalinine have been known for some time (Stadler et al., 1964), only recently have these alkaloids been reported as natural products (i.e., from cultures of *C. purpurea*) (Brunner et al., 1979).

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LITERATURE CITED

Agurell, S. *Acta. Pharm. Suec.* **1965**, *2*, 357.
Agurell, S.; Ramstad, E.; Ullstrup, A. *Planta Med.* **1963**, *11*, 392.
Arsenault, G. P. In "Biochemical Applications of Mass Spectrometry"; Waller, G. R., Ed.; Wiley-Interscience: New York, 1972.
Bacon, C. W.; Porter, J. K.; Robbins, J. D. *J. Gen. Microbiol.* **1979**, *113*, 119.
Barber, M.; Weisbach, J. A.; Douglas, B.; Dudek, G. O. *Chem. Ind. (London)* **1965**, 1072.
Bhattacharji, A.; Birch, A. J.; Brack, A.; Hofmann, A.; Kobel, H.; Smith, H.; Winter, J. *J. Chem. Soc.* **1962**, 421.
Brunner, R.; Stütz, P. L.; Tscherter, H.; Stadler, P. A. *Can. J. Chem.* **1979**, *57*, 1638.
Cassady, J. M.; Abou-Chaar, C. I.; Floss, H. G. *Lloydia* **1973**, *36*, 390.
Fales, H. M.; Lloyd, H. A.; Milne, G. W. A. *J. Am. Chem. Soc.* **1970**, *92*, 1590.
Fehr, T. Dissertation No. 3967, ETH, Zürich, 1967.
Fieser, L. F.; Fieser, M. "Reagents in Organic Synthesis"; Wiley: New York, 1967; Vol. I, p 247.
Groger, D.; Syring, U.; Johne, S. *Pharmazie* **1975**, *30*, 440.
Horwell, D. C.; Verge, J. P. *Phytochemistry* **1979**, *18*, 519.
Porter, J. K.; Bacon, C. W.; Robbins, J. D. *Lloydia* **1978**, *41*, 654.
Porter, J. K.; Bacon, C. W.; Robbins, J. D. *J. Agric. Food Chem.* **1979a**, *27*, 595.
Porter, J. K.; Bacon, C. W.; Robbins, J. D. *J. Nat. Prod.* **1979b**, *42*, 309, 696.
Porter, J. K.; Betowski, D. *J. Agric. Food Chem.* **1981**, preceding paper in this issue.
Schmidt, J.; Kraft, R.; Voigt, D. *Biomed. Mass Spectrom.* **1978**, *5*, 674.
Stadler, P. A.; Frey, A. J.; Ott, H.; Hofmann *Helv. Chim. Acta* **1964**, *47*, 1911.
Stadler, P. A.; Stutz, P.; Sturmer, E. *Experientia* **1977**, *33*, 1552.
Stahl, E. "Thin Layer Chromatography, a Laboratory Handbook"; Springer-Verlag: New York, 1969; No. 74, p 452.
Vokoun, J.; Rehacek, Z. *Collect. Czech. Chem. Commun.* **1975**, *40*, 1731.
Vokoun, J.; Sajdl, P.; Rehacek, Z. *Zentralbl. Bakteriol., Parasitenkd., Infektionskr. Hyg., Abt. 2, Naturwiss.: Allg., Landwirtsch. Tech. Mikrobiol.* **1974**, *129*, 499.
Yoneda, F.; Suzuki, K.; Nitta, Y. *J. Am. Chem. Soc.* **1966**, *88*, 2328.

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