LYSERGIC ACID AMIDE DERIVATIVES FROM *BALANSIA EPICHLOË* AND *BALANSIA CLAVICEPS* (*CLAVICIPITACEAE*)[1]

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Clavicipitaceous systemic phytopathogens, *Balansia epichloë* (Weese) Diehl, *B. henningsiana* (Möller) Diehl, *B. strangulans* (Mont.) Diehl, *Myriogenospora atramentosa* Berk. and Curt., and *Epichloë typhina* Fries, have been implicated in unexplained toxic syndromes occurring in cattle grazed on infected pasture grasses (1–5). The occurrence of the genus *Claviceps* and the role of ergot alkaloids in similar livestock problems have been well documented (6–9). The taxonomic relationships between *Balansia* and *Claviceps* (10, 11) and the association of *Balansia* with these "ergot-like" symptoms (1–5) prompted investigations into the possibility that *Balansia* may produce the ergoline-type alkaloids (3, 4, 12). Bacon *et al.* (13) demonstrated that *B. epichloë*, *B. strangulans*, *B. henningsiana*, and *B. claviceps* produced ergot alkaloids in laboratory cultures. We now report the chemical identification of the ergot alkaloids from *B. epichloë* as: chanoclavine I, isochanoclavine I, agroclavine, elymoclavine, penniclavine, ergonovine, ergonovinine, and two other clavine alkaloids (uv, m/e^{+}) that have molecular ions $M^{+}240$ and that do not correspond with festuclavine, pyroclavine, or costaclavine on co-chromatography. Also, we report on the isolation and identification of chanoclavine I, ergonovine, and ergonovinine from cultures of *B. claviceps*. Until now, no lysergic acid derivatives had been found in fungi outside the genus *Claviceps* (14).[2] The identification of ergonovine and ergonovinine from *B. epichloë* and *B. claviceps* suggests that other members of the Balansiae are capable of ergoline biosynthesis. Thus, members of the genus *Balansia* may have caused the unexplained incidences of "*Claviceps*- or ergot-like" syndromes in livestock that had ingested infected pasture grass.

EXPERIMENTAL[3, 4]

FUNGI.—*B. epichloë* (RRC 242) and *B. claviceps* (RRC 219) were cultured according to the two-stage fermentation procedure of Bacon *et al.* (13). The former organism was grown for 21 days in 50-ml of culture medium and for 36 days in 500 ml of medium contained in a 19–1 carboy. The latter was grown for 21 days as described (13).

EXTRACTION OF ALKALOIDS.—Mycelium and medium were separated by filtration with cheese cloth. The mycelium was stored at −10° for future studies. The medium was adjusted to pH 9–10 with NaOH (4N) and extracted with three equivalent volumes of $CHCl_3$. The extracts were combined, concentrated to ca. 100 ml (*in vacuo*, <30°) and washed with three 75-ml portions of 2% tartaric acid (w/v). The tartaric acid ex-

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[2]Note added in proof: The previously reported unknown N-substituted derivatives of lysergic acid amide isolated from *Epichloe typhina*, *Lloydia* **41**, 654–655 (1978), have been identified as ergosine and ergosinine, *J. Agr. Food Chem.* **27**, in press (1979).

[3]Melting points (mp) uncorrected, Mettler FP5 apparatus connected to a Mettler FP52 microfurnace; uv, Cary (model 15) MeOH; mass spectra, determined as direct insertion probe samples on a Hewlett-Packard (model 5930) dodecapole mass spectrometer at probe temperatures between 100–250°.

[4]Mention of firm names or trade products does not imply endorsement by the U.S. Department of Agriculture over other firms or similar products not mentioned.

tracts were combined, the pH was adjusted to 9–10 with NaOH (4N), and the basic solution extracted with $CHCl_3$ (3 x 100 ml). The $CHCl_3$ extracts were combined, dried over anhydrous Na_2SO_4, and concentrated as above to 3 ml.

CHROMATOGRAPHY.—Thin-layer chromatography (tlc) was performed with 20- x 20- and 20- x 40-cm glass plates coated with silica gel GF 254 (Brinkman) 0.25, 0.50, and 0.75 mm thick. Extracts and pure compounds, dissolved in either chloroform or chloroform-methanol (1:1, v/v), were applied with a Desaga autoliner (Brinkmann). Solvent systems (v/v) were: I. chloroform-methanol (90:10), II. chloroform-diethylamine (90:10); III. benzene-dimethylformamide (85.5:13.5); IV. chloroform-methanol (90:10) in a saturated ammonia atmosphere; and V. chloroform-t-butanol (3:1) in a saturated ammonia atmosphere according to reported procedures (1, 15–17). The plates were visually examined under uv at 254 and 366 nm and then after they had been sprayed (at the edge for preparative tlc) with N,N-*para*-dimethylaminobenzaldehyde (PDAB) (18). Those bands corresponding to a blue reaction (with PDAB) for the ergoline ring system were collected on fritted disc glass funnels and eluted from the silica gel with chloroform-methanol (1:1, v/v). The preparative plates were developed in solvent system I (25°); the bands corresponding to the blue reaction with PDAB were collected, eluted as described, and the eluate chromatographed in solvent system II (21°). The alkaloids were then eluted from the silica as before and were pure enough to be identified by uv and low resolution mass analyses. All identifications were based on their co-chromatography with authentic standards (1) in solvent systems I, II, and III (unless stated otherwise) and on uv (16, 19) and mass spectra (17, 20–22). Although literature values differed slightly from those obtained under the conditions of our mass spectral analyses (i.e. ion-intensities), no salient differences were observed between authentic materials and the alkaloids we isolated.

RESULTS AND DISCUSSION

Seven fractions (A–G) were separated from the alkaloid extract of the 21-day-old culture of *B. epichloë: Fraction A* appeared as a light blue band (R_f 0.00–0.03) under uv at 254 nm after tlc in solvent system I. It remained homogeneous in solvent system II, and its R_f (0.12) corresponded to that of chanoclavine I. However, when subjected to preparative tlc (22°C) in solvent system IV (17), this fraction separated into two compounds. One of them was chanoclavine I: R_f 0.26; mp 215 (lit 220°, 222°) (23, 24); λ max (MeOH) 292, 282, 275, 223 nm; m/e^+ 257 (17%, m+1), 256 (68%, M^+) 238 (24%), 237 (63%), 224 (8%), 223 (22%), 208 (14%), 207 (14%), 206 (24%), 197 (18%), 196 (26%), 184 (27%), 183 (100%), 182 (75%), 169 (25%), 168 (63%), 167 (63%), 166 (17%), 156 (37%), 155 (85%), 154 (95%), 153 (17%), 144 (19%), 131 (15%), 130 (35%), 128 (24%), 127 (43%), 116 (10%), 115 (35%), 108 (27%), 102 (24%), 101 (45%). The other compound (R_f 0.19) gave a positive blue reaction with PDAB, but it could not be identified because of the small quantity available.

Fraction B appeared as a light blue band (R_f 0.08–0.12) at 254 nm after separation by solvent system I. It was shown by further tlc-m/e^+ analyses to be a mixture of chanoclavine I and two other compounds (R_f 0.36 and R_f 0.19, system IV), both of which gave a positive blue reaction to PDAB. One compound (R_f 0.36) qualitatively gave uv and mass analyses identical to those of chanoclavine I and was tentatively identified as isochanoclavine I. This identification was based on m/e^+ 256 (M^+), 237, 183, 168, 167, 155, 154, and the results obtained by Cassady *et al.* (17) for the separation of chanoclavines I, II, and isochanoclavine I in solvent systems IV and V. On the basis of tlc, the compound with R_f 0.19 appeared to be the same unknown material isolated in fraction A; however, sample quantity prevented us from determining whether the compound was chanoclavine II which, like other compounds, has similar R_f values to those of the unknown in systems IV and V (17).

Fraction C was not visible in uv after tlc in solvent system I (R_f 0.15–0.21). It was separated by solvent

system II into two compounds, one of which was elymoclavine: R_f 0.10; λ max (MeOH) 292, 282, 275, 220 nm; *m/e*$^+$ 255 (12%, M+1), 254 (81% M$^+$), 253 (100%, M−1), 238 (3%), 237 (6%), 224 (3%), 223 (12%), 207 (6%), 206 (5%), 205 (5%), 193 (5%), 192 (9%), 191 (5%), 181 (6%), 180 (11%), 168 (8%), 167 (15%), 155 (7%), 154 (21%), 140 (4%), 139 (8%), 128 (10%), 127 (17%). The other compound (R_f 0.50) gave a blue PDAB reaction, and its uv (λ max (MeOH) 292, 280, 272, 220 nm) and mass spectrum (figure 1) were characteristic of a clavine alkaloid (21). Tlc comparison with authentic standards (chloroform-methanol, 4:1, v/v) proved this compound different from pyroclavine, costaclavine, and festuclavine. This compound was labeled B.e. alkaloid no. 1, and structure identification is the subject of future studies.

Fraction D, a pale blue band (R_f 0.28–0.32) at 254 and 366 nm after tlc in solvent system I, remained homogeneous in system II and corresponded to ergonovine: R_f 0.02; λ max, (MeOH) 312, 242 nm; *m/e*$^+$ 326 (26%, m+1), 325 (100%, M$^+$), 268 (8%), 267 (17%), 224 (13%), 223 (39%), 222 (30%), 221 (74%), 220 (17%), 208 (23%), 207 (59%), 197 (8%), 196 (54%), 182 (12%), 181 (46%), 180 (24%), 168 (12%), 167 (23%), 155 (7%), 154 (27%).

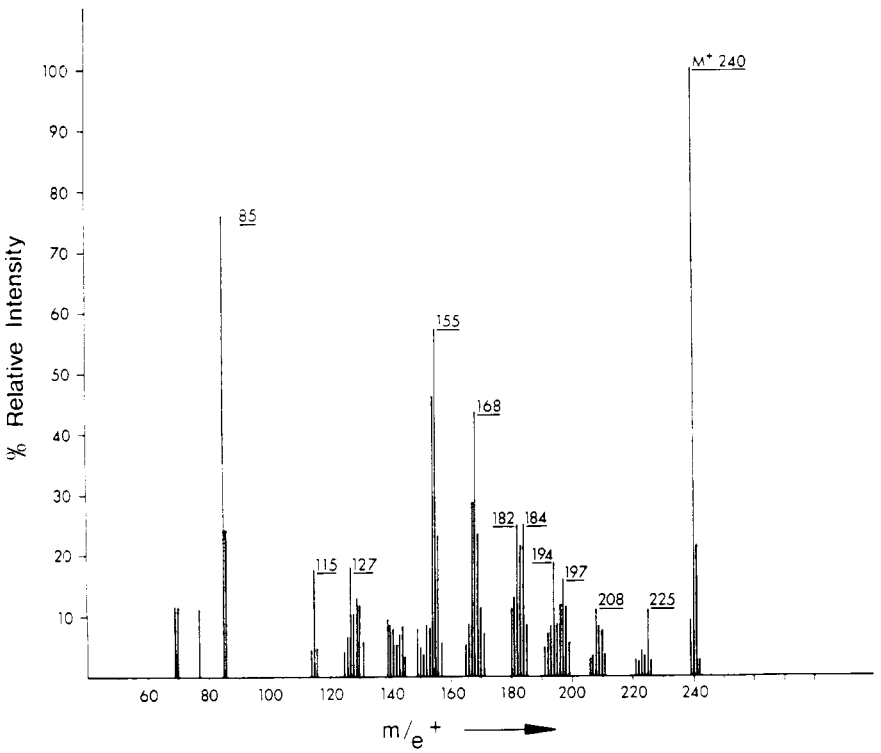

FIG. 1. *Mass spectrum B.e. alkaloid no. 1.*

Fraction E, a pale blue band (R_f 0.46–0.50) at 254 and 366 nm after tlc in solvent system I, was separated into two compounds by system II. One was ergonovinine: R_f 0.20; λ max

(MeOH) 310, 242 nm; m/e^+ (as ergonovine). The other compound was agroclavine: R_f 0.48, λ max (MeOH) 292, 280, 275, 222 nm; m/e^+ 239 (3%, m+1), 238 (60%, M^+), 237 (100%, M−1), 224 (2%), 223 (13%), 222 (9%), 221 (9%), 220 (5%), 208 (5%), 207 (6%), 206 (6%), 205 (5%), 193 (5%), 192 (7%), 191 (6%), 183 (6%), 182 (8%), 181 (11%), 180 (14%), 168 (10%), 167 (22%), 166 (10%), 155 (9%), 154 (33%), 153 (6%), 128 (10%), 127 (27%), 126 (10%), 119 (9%), 118 (26%), 115 (15%), 111 (24%), 110 (12%), 109 (6%), 108 (27%).

Fraction F, which appeared as a dark blue band (R_f 0.55–0.58) at 254 and 366 nm after tlc in solvent system I, was separated by system II into two compounds that were visible at 254 and 366 nm and that gave a positive reaction (blue) with PDAB. One corresponded to ergonovinine (tlc, uv, m/e^+) while the other compound (R_f 0.23) was isolated in quantities sufficient only for uv analyses (λ max (MeOH) 307, 237 sh) and is unidentified. Mass data of the mixture F isolated after tlc in solvent system I showed in addition to M^+325 for ergonovinine, ion fragments occurring at m/e^+ 370, 369, 368, 367 and 354, 353.

Fraction G was not visible in uv after tlc in solvent system I (R_f 0.72–0.76). Before chromatography in system II, this fraction exhibited an ultraviolet maximum at 355 nm, shoulders centered at 292 and 275 nm, ion fragments at m/e^+ 368, 356, and 310, with typical tricyclic and tetracyclic ergoline fragments at m/e^+ 256, 221, 206, 194, 192, 155, and 154 (17, 20, 21). This compound was not observed after chromatography in solvent system II and thus was not studied further.

B. epichloë, incubated for 36 days, produced all the above identified alkaloids plus several others. Agroclavine and elymoclavine were produced in trace amounts, as determined by tlc. Penniclavine was present (color reaction green with PDAB): λ max (MeOH) 314, 242 nm; m/e^+ 271 (12%, M+1), 270 (64%, M^+), 237 (10%), 236 (10%), 227 (21%), 221 (12%), 209 (26%), 208 (43%), 196 (40%), 192 (16%), 181 (46%), 168 (30%), 167 (20%), 155 (38%), 154 (100%). Also produced was another clavine alkaloid (designated B.e. alkaloid no. 2), which turned yellow-green with PDAB, was visible at 366 nm, λ max (MeOH) 312, 242 nm, and gave an apparent molecular ion of m/e^+ 240 (figure 2). Penniclavine and B.e. alkaloid no. 2 were isolated from the ergonovine and ergonovinine fractions respectively by preparative tlc in system II, followed by tlc in methylene chloride-2-propanol (90:10, v/v). Furthere studies on B.e. alkaloid no. 2 will be reported later. Total alkaloid production (spectrophotometrically determined as ergonovine maleate) for *B. epichloë* grown in 500 ml of culture medium for 36 days was 390 mg/liter. No attempt was made to quantitate the individual alkaloids, however the chanoclavine fraction visually appeared (color reaction with PDAB, tlc) to be the major alkaloids produced by *B. epichloë*.

The alkaloid extract from *B. claviceps* (190 mg/liter, spectrophotometrically determined as ergonovine maleate) contained three compounds that gave a blue reaction with PDAB. Ergonovine and chanoclavine I were identified on the basis of uv, tlc, and mass analyses (also chanoclavine I co-chromatographed with authentic chanoclavine I in solvent systems IV and V). Ergonovinine isolated in limited quantity was identified on the basis of its co-chromatography with authentic compound in chloroform-methanol (80:20, v/v) and methylene chloride-2-propanol (90:10, v/v).

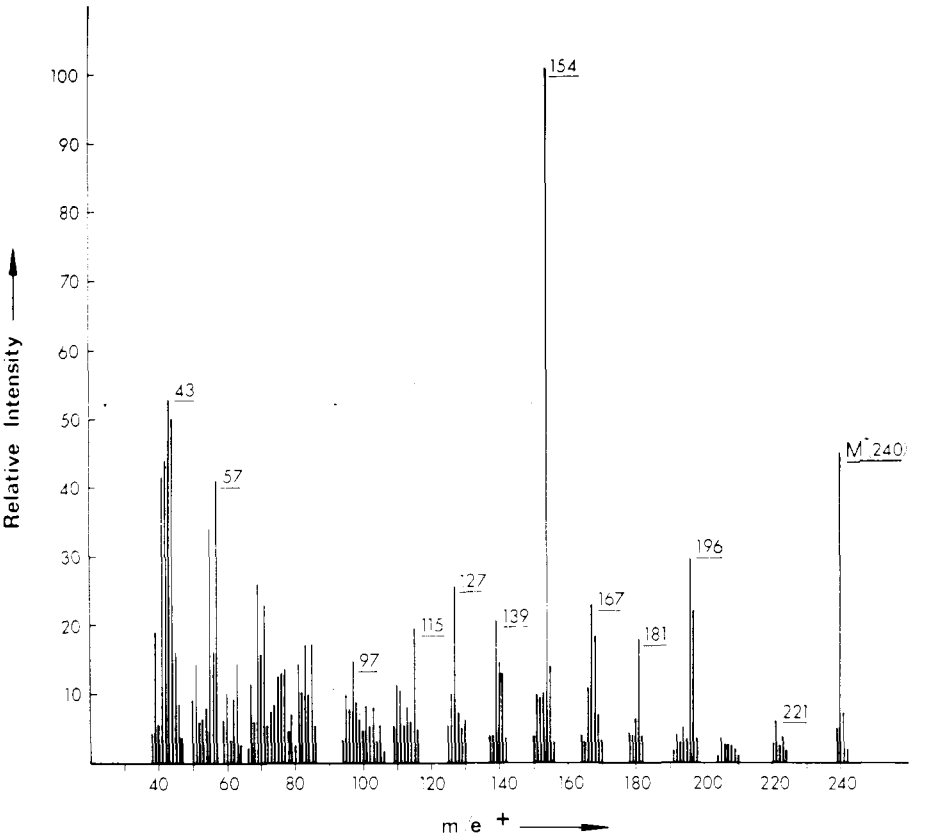

FIG. 2. *Mass spectrum B.e. alkaloid no. 2.*

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