

activation of spores, the red-far-red induction phenomenon, or the sensing of an odor or an insect pheromone, rather than a metabolic shift in which stimulator molecules (i.e. ionone or nonanal) are continuously required as substrate for a new branch in a metabolic pathway. In simpler terms, the stimulator molecules appear to trigger a very sensitive chemical switch, rather than function as an additional energy source for a new branch of the metabolic circuit.

The rapid response of spores to stimulatory volatiles suggests that brief exposures might have some practical value in inducing spores to germinate under circumstances which would preclude infection of the host and hence prevent the disease. The results of short-time exposure to stimulators, interacting with water vapor and the endogenous germination inhibitors described by Allen (1955) and identified by Macko et al. (1970-1972), are being studied in detail to determine possible applications in spore germination control and hence fungal disease control.

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The authors are indebted to John F. Brown, The University of New England, Armidale, N.S.W., Australia for reference to stimulation of spores of *Urocystis tritici* Koern. by benzaldehyde and related compounds, reported by R. J. Noble (1924).

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## Indole Alkaloids from *Balansia epichloë* (Weese)

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*Balansia epichloë* (Weese) is a clavicipitaceous fungus which parasitizes pasture grasses. This class of fungi may be involved with ergot-type syndromes observed in cattle grazed on infected pastures. Three indole alkaloids were isolated from laboratory cultures of *B. epichloë* and their identities were determined by ultraviolet, infrared, nuclear magnetic resonance, and mass spectroscopy as: 4-(3-indolyl)butane-1,2,3-triol; 3-(3,3-diindolyl)propane-1,2-diol; and 3-(3-indolyl)propane-1,2,3-triol. The alkaloids were toxic to fertile leghorn eggs.

*Balansia epichloë* (Weese), a systemic grass pathogen, has been implicated in ergot-type syndromes observed in cattle grazed on pasture grasses (Bacon et al., 1975; Nobindro, 1934; Porter et al., 1975). Bermuda grass tremors (convulsive ergotism) and fescue foot (gangrenous ergotism) are associated with cattle grazed on *Cynodon dactylon* (L.) Pers and *Festuca arundinaceae* Schreb., respectively.

The indications that *Balansia* may be involved in the etiology of clavicipitaceous diseases in cattle prompted us to investigate the possibility of indole alkaloid production by *Balansia*. From submerged cultures of an isolate of *B. epichloë* (Bacon et al., 1975) two fractions (A and B) were isolated by preparative thin-layer chromatography (TLC). Each gave a UV spectrum (Bacon et al., 1975; Agurell, 1965) and color reaction with *p*-dimethylaminocinnamaldehyde (Stahl, 1969) characteristic for indole alkaloids. We report the isolation, chemical characterization, and

toxicities of three compounds in these fractions.

#### EXPERIMENTAL SECTION

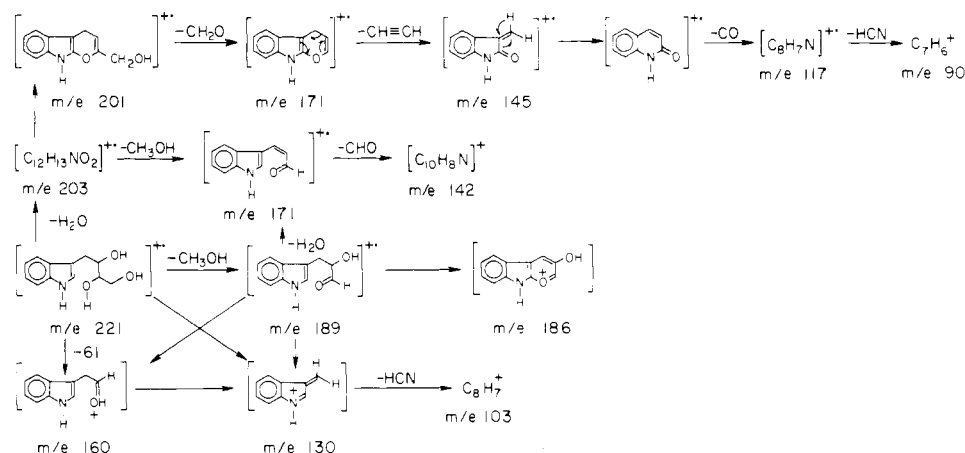
**Materials and Methods of Culture.** Subcultures and inocula of *B. epichloë*, 200 SF, were prepared according to Bacon et al. (1975). The fungus was incubated in a 2.8-l. Fernback flask for 21 days at 24 to 28 °C, on a gyratory shaker (200 rpm, 1-cm circular orbit) in 250 ml of the following medium: soluble starch (2% solution, pH 5.8), 70 g; mannitol, 30 g; ammonium succinate, 12 g; MgSO<sub>4</sub>·7H<sub>2</sub>O, 2 g; K<sub>2</sub>SO<sub>4</sub>, 0.70 g; FeSO<sub>4</sub>·7H<sub>2</sub>O, 50 mg; CuSO<sub>4</sub>·5H<sub>2</sub>O, 5 mg; ZnSO<sub>4</sub>·7H<sub>2</sub>O, 5 mg; MnSO<sub>4</sub>·H<sub>2</sub>O, 10 mg; thiamin, 0.2 µg/ml; distilled water, 1000 ml; and concentrated NH<sub>4</sub>OH added to adjust the pH to 5.6.

**Chromatography.** Column chromatography was performed on Porapak Q (80/100 mesh; Waters Associated Inc.) as previously described (Bacon et al., 1975). Preparative TLC was on silica gel GF 254 (Brinkman) according to reported procedures (Bacon et al., 1975; Porter et al., 1974) and the developing solvent systems were (v/v): (I) CHCl<sub>3</sub>-CH<sub>3</sub>OH (80:20); (II) C<sub>6</sub>H<sub>6</sub>-DMF (86.5:13.5). All solvents were analytical reagent grade and were not further purified.

**Analytical Methods.** Ultraviolet (UV) spectra of the

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Scheme I



samples in methanol (reagent grade) were determined with a Varian Cary 15 spectrophotometer. Infrared (IR) spectra were obtained from micro-KBr pellets with a Perkin-Elmer 457A grating infrared spectrophotometer. Low-resolution mass spectral data were determined via direct insertion probe samples (source 275 °C) on a DuPont 21492 mass spectrometer. High-resolution data were obtained from the High Resolution Mass Spectrometry Laboratory, Florida State University, Tallahassee, Fla. Pulsed NMR data were obtained in CD<sub>3</sub>OD on a JEOL PS/PFT 100 NMR spectrometer connected with a Nicolet 1083 computer system.

**Toxicity Studies.** Compounds were dissolved in water and bioassayed for toxicity with fertile eggs of white leghorns according to Verrett et al. (1964). Each dosage was tested on an average of two replications; each replication consisted of 15 or 20 eggs unless otherwise stated. Toxicity was defined as an inability of eggs to hatch after 24 days of incubation. Mortality for all controls was zero.

**Synthesis. 3-(3,3-Diindolyl)propane-1,2-diol.** Indole (0.3 mM) in 1 ml of 95% C<sub>2</sub>H<sub>5</sub>OH was added dropwise (2–3 min) to a chilled solution of glyceraldehyde (0.3 mM) in 1 ml of HCl (0.1 N)–95% C<sub>2</sub>H<sub>5</sub>OH (30:70, v/v). The reaction mixture was allowed to stand for 1.5 h (ice bath) and then at room temperature (2 h). The solution was heated (screw cap Reacti-vial, Pierce) at 50 °C (1 h), allowed to stand at room temperature for 15 h, and rendered basic (pH 8–9) with 2% ammoniacal C<sub>2</sub>H<sub>5</sub>OH. Preparative TLC in solvent systems I and II as described (cf. text) yielded 24 mg of 3-(3,3-diindolyl)propane-1,2-diol identical with the natural compound as determined by UV, M<sup>+</sup>, TLC, and reported IR spectra (Lingens and Goebel, 1967).

**3-(3-Indolyl)propane-1,2,3-triol.** Glyceraldehyde (0.3 mM) and indole (0.3 mM), each in 200 μl of CH<sub>3</sub>OH, were reacted at room temperature. After 1 h, trace amounts of a compound analogous to natural diol A-2 (cf. text) were observed by TLC in solvent systems I and II. The reaction mixture was heated (65 °C) in a screw cap Reacti-vial (Pierce) for 20 h. By preparative TLC sufficient quantities of 3-(3-indolyl)propane-1,2,3-triol were isolated for UV and M<sup>+</sup> analysis. The UV and M<sup>+</sup> data were identical with the natural compound B (M<sup>+</sup> 207, cf. text) and data reported for this compound (Preobrazhenskaya et al., 1965). 3-(3,3-Diindolyl)propane-1,2-diol was isolated as the major compound.

**Semisynthetic 3-(3,3-Diindolyl)propane-1,2-diol from Natural 3-(3-Indolyl)propane-1,2,3-triol (B, M<sup>+</sup> 207).** A methanolic solution (500 μl) of compound B (0.01 mM) was heated (65 °C) with indole (0.01 mM) in a screw cap reacti-vial (1.5 h) and allowed to stand at room temper-

Table I. Ultraviolet Absorption of *B. epichloë* Alkaloids

Compd	$\lambda_{\max}(\text{CH}_3\text{OH})$ (log $\epsilon$ ), nm
A-1	221 (4.653), 272 (3.784), 279 (3.800), 288 (3.734)
A-2	221 (4.883), 275 (3.978), 282 (4.019), 291 (3.968)
B	220 (4.958), 273 (4.000), 280 (4.020), 289 (3.952)

ature 16 h. TLC in solvent system I as described showed the diol reaction product (minor) analogous to natural A-2 and starting material (major) natural B. Trace amounts of 0.1 N HCl were added and the mixture heated (2 h) with periodic agitation. Preparative TLC (solvent systems I and II) demonstrated only starting indole and a compound identical with 3-(3,3-diindolyl)propane-1,2-diol by UV and M<sup>+</sup> analysis.

## RESULTS AND DISCUSSION

**Alkaloid Isolation and Characterization.** Indole alkaloid fractions A and B (Bacon et al., 1975) were isolated by preparative TLC on silica gel in solvent system I (A, *R<sub>f</sub>* 0.67; B, *R<sub>f</sub>* 0.45). Fraction A when subjected to TLC in solvent system II separated into two compounds, A-1 (*R<sub>f</sub>* 0.43) and A-2 (*R<sub>f</sub>* 0.25). Compound B remained homogeneous in this system (*R<sub>f</sub>* 0.17). After elution of the compounds from the silica gel (chloroform–methanol, 1:1, v/v), rechromatography of these three compounds in system I as described (Bacon et al., 1975) did not change their *R<sub>f</sub>* values (A-1, *R<sub>f</sub>* 0.67; A-2, *R<sub>f</sub>* 0.67; B, *R<sub>f</sub>* 0.45).

Compounds A-1 and A-2 gave the UV spectra (Table I) and color reaction with *p*-dimethylaminocinnamaldehyde (A-1, red-violet; A-2, blue; B, red-violet) characteristic for indole alkaloids (Brown et al., 1952; Stahl, 1969).

Low-resolution mass data indicated that compounds A-1 and A-2 were homogeneous systems with molecular weights of 221 and 306, respectively (Figure 1). Fraction B appeared as a mixture of two compounds with molecular weights of 207 (major) and 378 (minor). High-resolution mass data (Tables II and III) established the following molecular formulas: A-1, C<sub>12</sub>H<sub>15</sub>NO<sub>3</sub>; A-2, C<sub>19</sub>H<sub>18</sub>N<sub>2</sub>O<sub>2</sub>; B, C<sub>11</sub>H<sub>13</sub>NO<sub>3</sub> and C<sub>22</sub>H<sub>22</sub>N<sub>2</sub>O<sub>4</sub>. All three spectra (Figure 1) had a major mass peak at *m/e*<sup>+</sup> 130 (Table II) which indicated cleavage (Scheme I) β to a 3-substituted indole nucleus (Biemann et al., 1961; Budzikiewicz et al., 1964a, 1972; Jamison and Hutzinger, 1970; Powers, 1968). The UV data for A-1, A-2, and B (Table I) are also consistent with simple 3-substituted indoles (Brown et al., 1952; Szmuszkovicz, 1962). The presence of such indoles was also supported by the strong IR bands (Figure 2) at 1080

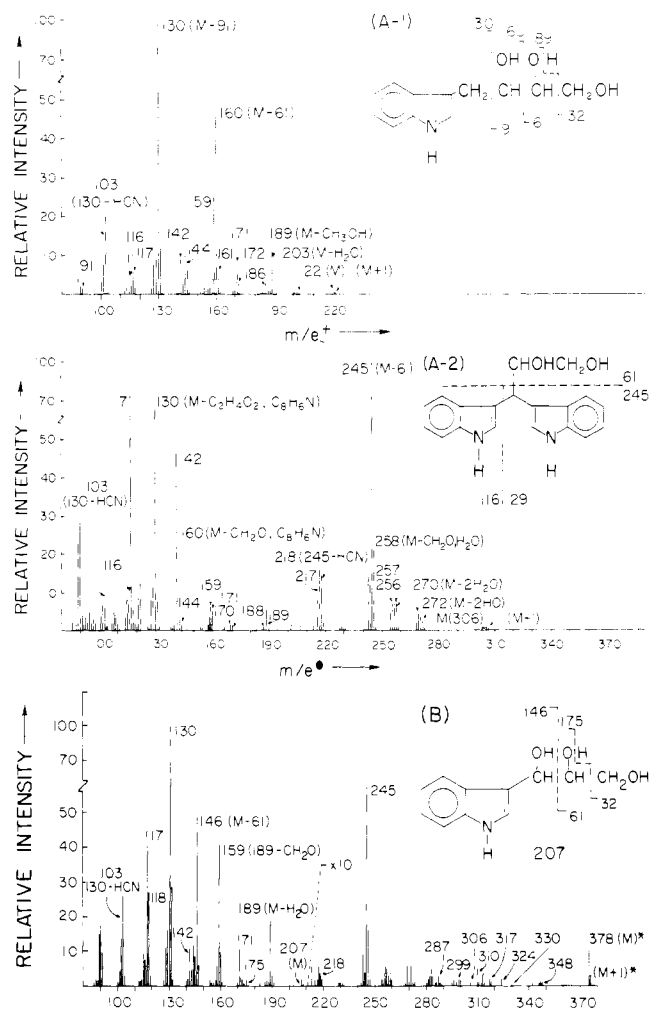


Figure 1. Mass spectra of *Balansia* alkaloids A-1, A-2, and B.

(A-1) and  $1085\text{ cm}^{-1}$  (A-2 and B) and by the weak absorptions at  $1550$ ,  $1410$ , and  $780\text{ cm}^{-1}$  (Brown et al., 1952). Strong absorptions at  $1420$  and  $740\text{ cm}^{-1}$  were consistent with the indole moiety (Brown et al., 1952).

The 3-substituted indole moiety was further confirmed by a comparison of their  $^1\text{H}$  NMR spectra with simple indole in  $\text{CD}_3\text{OD}$ . All three compounds showed no absorption at  $\delta$  6.40–6.45 (Figure 3) consistent with the absence of the proton in the 3 position of the indole nucleus (Black and Hefferman, 1965).

The broad bands in the infrared spectra (Figure 2) at  $1030$  (A-1),  $1050$  (A-2), and  $1065\text{ cm}^{-1}$  (B) were assigned the C–O stretching vibrations of the side-chain alcohols (Colthap et al., 1965). This assignment is supported by bands at  $1340$  (A-1 and A-2) and  $1335\text{ cm}^{-1}$  (B), vibrations for primary and secondary alcohols (Dyer, 1965). Loss of 61 mass units ( $M - 61$ , Figure 1, Table II) in A-1 ( $m/e$  160,  $\text{C}_{10}\text{H}_{10}\text{NO}$ ) and A-2 ( $m/e$  245,  $\text{C}_{17}\text{H}_{13}\text{N}_2$ ) was attributed to loss of  $\text{CHOHCH}_2\text{OH}$  and is characteristic for diol systems (Budzikiewicz et al., 1964b). The analogous fragmentation was observed in the spectrum of B (Figure 1) which enabled assignment of ions  $m/e$  146 ( $207 - 61$ ,  $\text{C}_9\text{H}_8\text{NO}$ ) (Table II) and  $m/e$  317 ( $378 - 61$ ,  $\text{C}_{20}\text{H}_{17}\text{N}_2\text{O}_2$ ) (Table III).

In the spectrum of compound A-1, the presence of such a diol group would result in fragments at  $m/e$  203 ( $M - \text{H}_2\text{O}$ ), 202, 201, 189 ( $M - \text{CH}_3\text{OH}$ ), 171 ( $189 - \text{H}_2\text{O}$ ), 160 ( $M - 61$ ), 159, 144 ( $203 - \text{COCH}_2\text{OH}$ ), 142 ( $171 - \text{CHO}$ ), 103 ( $130 - \text{HCN}$ ), 91 ( $M - 130$ ), 90 ( $117 - \text{HCN}$ ), and 89 (Scheme I, Figure 1). Formation of  $m/e$  145 ( $\text{C}_9\text{H}_7\text{NO}$ ,

Table II) in A-1 may be explained through the fragmentation of the enol  $m/e$  203 (Scheme I). High-resolution measurements (Table II) support the proposed pathway for the tentative identification of A-1 as 4-(3-indolyl)-butane-1,2,3-triol.

In A-2, ion  $m/e$  245 ( $M - 61$ ) appears to have been formed by  $\beta$  cleavage (Scheme II) to the indole nucleus (Biemann et al., 1961; Budzikiewicz et al., 1964a, 1972; Jamison and Hutzinger, 1970; Powers, 1968). This cleavage is supported by the peak (Figure 1) at  $m/e$  218 ( $245 - \text{HCN}$ ;  $\text{C}_{16}\text{H}_{12}\text{N}$ ) and  $m/e$  217 ( $\text{C}_{16}\text{H}_{11}\text{N}$ ) (Table II) (Budzikiewicz et al., 1964b). Indolyindole attachment ( $2,2'$ ;  $3,3'$ ; or  $2,3'$ ) for A-2 was eliminated on the basis of its UV absorption (Fasseh and Harley-Mason, 1957; Young, 1962). The diol system  $\text{CHOHCH}_2\text{OH}$  in A-2 also gave the minor peaks expected at  $m/e$  272 ( $\text{C}_{19}\text{H}_{16}\text{N}_2$ ), 270 ( $M - 2\text{H}_2\text{O}$ ;  $\text{C}_{19}\text{H}_{14}\text{N}_2$ ), 258 ( $M - \text{CH}_2\text{O}$ ,  $\text{H}_2\text{O}$ ;  $\text{C}_{18}\text{H}_{14}\text{N}_2$ ), 257 ( $\text{C}_{18}\text{H}_{13}\text{N}_2$ ), and 256 ( $\text{C}_{18}\text{H}_{12}\text{N}_2$ ). Proton transfer (Scheme II) from the primary hydroxyl to the methine carbon or the indoly moiety with loss of indolyl as  $m/e$  116 or  $m/e$  117, respectively, and loss of  $\text{CH}_2\text{O}$  would result in the observed  $m/e$  160, 159, 142 ( $258 - \text{C}_8\text{H}_6\text{N}$ ), 130, and fragments analogous to those formed in A-1 (Figure 1).

Generation of ion  $m/e$  189 through the loss of indolyl as  $m/e$  117 may also explain the formation of  $m/e$  159 and 130. Although the  $m/e$  189 ion calculates for the  $m + 1$  peak ( $189.0745$ ) of  $m/e$  188 (Table II), its relative intensity (6%) is inconsistent for this fragment contributing solely to the isotopic moiety. Ion  $m/e$  188 may be formed by either  $189 - \text{H}$  or by the loss of indolyl as  $M - 117$  (Scheme II) through  $\beta$  cleavage (the preferred pathway) with subsequent cyclization ( $m/e$  188,  $\text{C}_{11}\text{H}_{10}\text{NO}_2$ ). This pathway would lead to fragments  $m/e$  170 ( $188 - \text{H}_2\text{O}$ ;  $\text{C}_{11}\text{H}_8\text{NO}$ ) and  $m/e$  144 ( $\text{C}_9\text{H}_6\text{NO}$ ) analogous to that formed in A-1 (Scheme I).

The high-resolution data (Table II) and spectral interpretation of compound A-2 are consistent with 3-(3,3-diindolyl)propane-1,2-diol. Synthesis of A-2 by a minor modification of reported procedures (Lingens and Goebel, 1967) proved the natural and synthetic materials identical (UV, TLC,  $M^+$ ).

A major ion at  $m/e$  146 (Figure 1) in the mass spectrum of B was attributed to  $\text{C}_9\text{H}_8\text{NO}$  (Table II) resulting from  $207 - 61$  ( $\text{CHOHCH}_2\text{OH}$ ) as previously described (Scheme III). The fragmentation of  $m/e$  146 analogous to the mechanism proposed for the elimination of HCN from ions  $m/e$  245 and  $m/e$  130 (Schemes I and II; Budzikiewicz et al., 1964b) explains the intensity of fragment  $m/e$  118 (28%) for  $\text{C}_8\text{H}_8\text{N}$  (Table II and Scheme III) in spectra B (Figure 1). High-resolution mass data (Table II) of the ions occurring at  $m/e$  189, 175, 159, 130, 117, 103, 91, and 90 are consistent with the proposed pathway (Scheme III) for the fragmentation of 3-(3-indolyl)propane-1,2,3-triol. Synthesis of B ( $M^+$  207) by the action of glyceraldehyde and indole proved the natural and synthetic material identical (UV, TLC,  $M^+$ ). Also, the reaction of indole and natural B yielded the semisynthetic compound identical with natural A-2 and the synthetic material described above.

Predicated on indole chemistry (Noland and Venkiteswaran, 1961) it is tempting to speculate that the molecular ion at  $M^+$  378 in the mass spectrum of B ( $M^+$ , Figure 1) is the structure that would result from the cyclizative condensation of glyceraldehyde with 3-(3,3-diindolyl)propane-1,2-diol (A-2) at the  $2,2'$  positions. These structures are supported by the fragment ions observed at  $m/e$  348 ( $M^+ - \text{CH}_2\text{O}$ ), 347 ( $M^+ - \text{CH}_2\text{OH}$ ), 346 ( $M^+ - \text{CH}_3\text{OH}$ ), 330 ( $348 - \text{H}_2\text{O}$ ), 324 ( $M^+ - 3\text{H}_2\text{O}$ ), 317 ( $M^+$

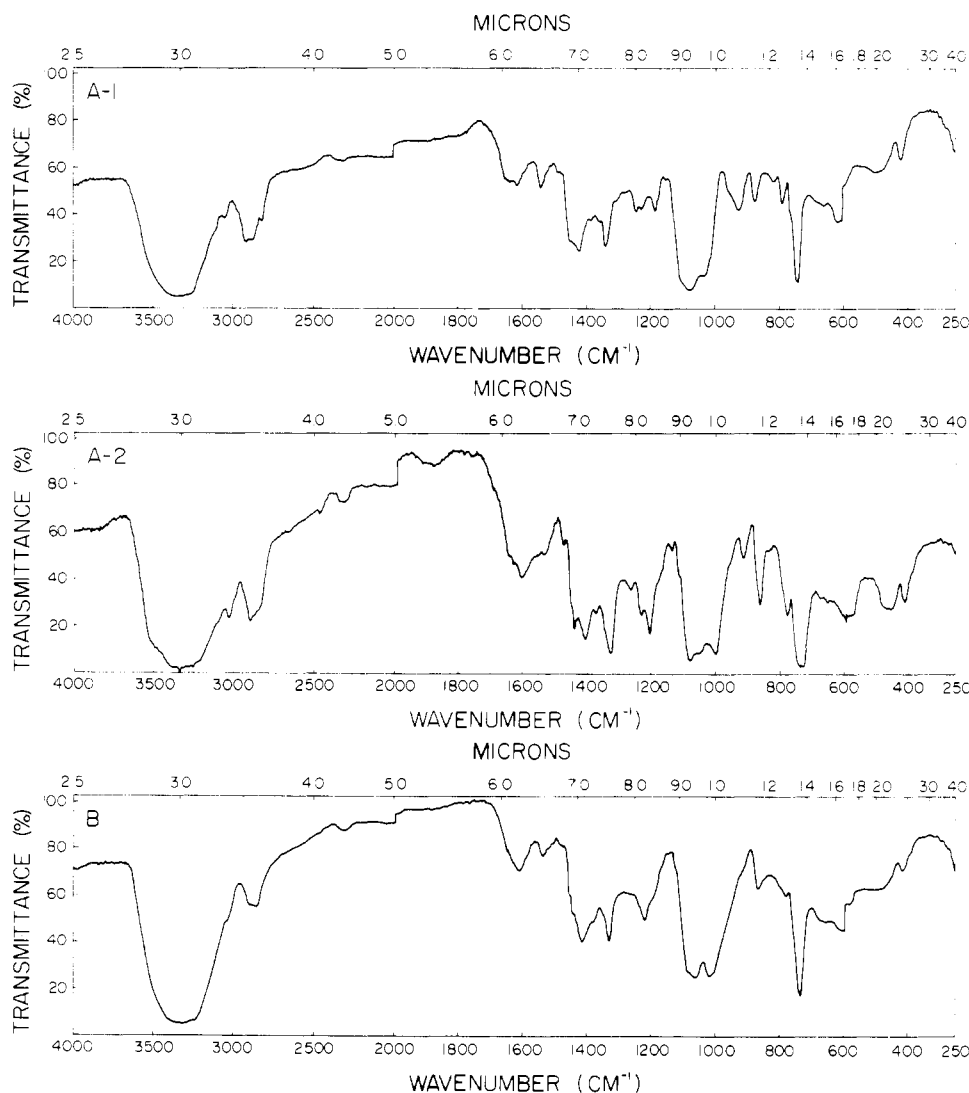
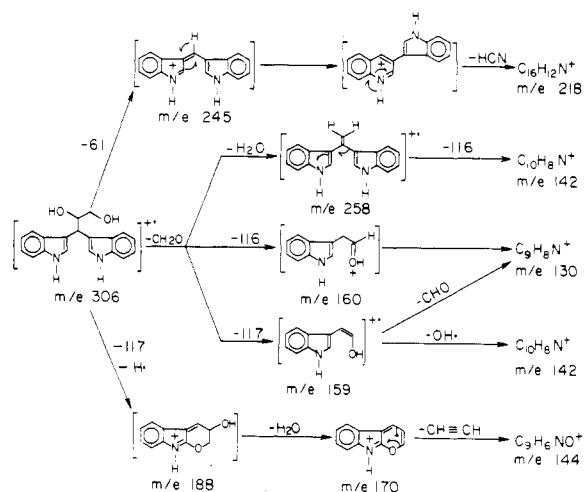


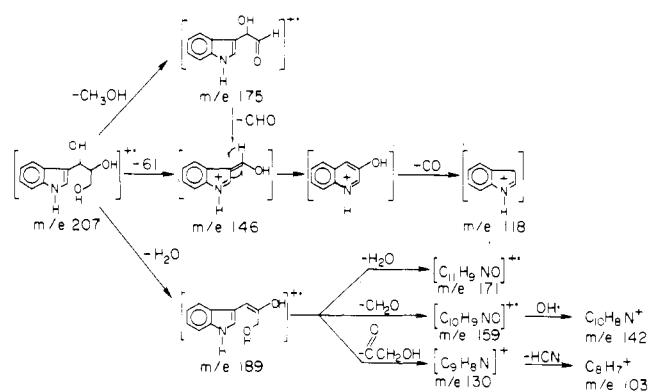
Figure 2. Infrared spectra (KBr) of *Balansia* alkaloids A-1, A-2, and B.

Scheme II



- 61), 310 (346 - 2H<sub>2</sub>O), 299 (317 - H<sub>2</sub>O), 287 (348 - 61), 281 (299 - H<sub>2</sub>O and/or 310 - 29), 280 (281 - H), and the high-resolution calculations for these masses (Table III). It is unknown at present if the ions *m/e* 306, 245, and 218 in the spectrum of B are a result of trace quantities of natural A-2 or the result of 3-indoleglycerol self-condensation. However, these fragment ions may be ra-

Scheme III



tionalized as occurring from  $M^+$  378. Since this compound appeared in such minor quantities in the spectrum of B, further investigations are needed in order to define these fragments.

Several of the proposed minor fragments for the alkaloids were isotopic with calculated masses for isotopic moieties of large fragments. In almost all cases, the intensities of their peaks were considerably higher than those calculated intensities for the expected isotopic moieties of the major fragments; however, their inclusion is consistent with the mechanistic rationale.

Table II. High-Resolution Mass Data of *Balansia epichloë* Alkaloids: A-1 (221), A-2 (306), and B (207)

Elem. composition	Calcd mass	Measd mass		
		A-1	A-2	B
C <sub>19</sub> H <sub>16</sub> N <sub>2</sub> O <sub>2</sub>	306.1368		306.1368 (M)	
C <sub>19</sub> H <sub>16</sub> N <sub>2</sub>	272.1313		272.1326	
C <sub>19</sub> H <sub>14</sub> N <sub>2</sub>	270.1156		270.1145	
C <sub>18</sub> H <sub>14</sub> N <sub>2</sub>	258.1156		258.1132	
C <sub>18</sub> H <sub>12</sub> N <sub>2</sub>	257.1077		257.1049	
C <sub>18</sub> H <sub>12</sub> N <sub>2</sub>	256.0999		256.0993	
C <sub>17</sub> H <sub>12</sub> N <sub>2</sub>	245.1078		245.1069	
C <sub>12</sub> H <sub>15</sub> NO <sub>3</sub>	221.1052	221.1049 (M)		
C <sub>16</sub> H <sub>12</sub> N	218.0969		218.0958	
C <sub>16</sub> H <sub>11</sub> N	217.0891		217.0887	
C <sub>11</sub> H <sub>13</sub> NO <sub>3</sub>	207.0894			207.0876 (M)
C <sub>12</sub> H <sub>13</sub> NO <sub>2</sub>	203.0945	203.0942		
C <sub>12</sub> H <sub>11</sub> NO <sub>2</sub>	201.0789	201.0800		
C <sub>11</sub> H <sub>11</sub> NO <sub>2</sub>	189.0789	189.0787		189.0765
C <sub>11</sub> H <sub>10</sub> NO <sub>2</sub>	188.0711	188.0715	188.0671	188.0680
C <sub>11</sub> H <sub>8</sub> NO <sub>2</sub>	186.0554	186.0550		186.0547
C <sub>11</sub> H <sub>10</sub> NO	172.0762	172.0743		172.0741
C <sub>11</sub> H <sub>9</sub> NO	171.0683	171.0687	171.0675	171.0686
C <sub>11</sub> H <sub>8</sub> NO	170.0605	170.0605	170.0628	170.0599
C <sub>10</sub> H <sub>10</sub> NO	160.0762	160.0750	160.0740	160.0721
C <sub>10</sub> H <sub>9</sub> NO	159.0684	159.0676	159.0673	159.0677
C <sub>9</sub> H <sub>8</sub> NO	146.0605	146.0595		146.0596
C <sub>9</sub> H <sub>8</sub> NO	145.0527	145.0527		145.0521
C <sub>10</sub> H <sub>10</sub> N	144.0812	144.0819		144.0803
C <sub>9</sub> H <sub>8</sub> NO	144.0449	144.0457	144.0429	144.0448
C <sub>10</sub> H <sub>9</sub> N	142.0656	142.0661	142.0634	142.0648
C <sub>9</sub> H <sub>8</sub> N	130.0656	130.0626	130.0661	130.0624
C <sub>8</sub> H <sub>8</sub> N	118.0656	118.0634	118.0620	118.0651
C <sub>8</sub> H <sub>7</sub> N	117.0578	117.0589	117.0573	117.0581
C <sub>8</sub> H <sub>6</sub> N	116.0500	116.0528	116.0515	116.0522
C <sub>8</sub> H <sub>7</sub>	103.0547	103.0535	103.0543	103.0545
C <sub>7</sub> H <sub>7</sub> O <sub>3</sub>	91.0395	91.0400		
C <sub>7</sub> H <sub>7</sub>	91.0547	91.0529	91.0531	91.0526
C <sub>7</sub> H <sub>6</sub>	90.0469	90.0446	90.0465	90.0450
C <sub>7</sub> H <sub>5</sub>	89.0391	89.0382	89.0393	89.0377

Table III. High-Resolution Mass Data for *Balansia* Alkaloid B (M<sup>+</sup> 378)\*

Elem. composition	Calcd mass	Measd mass
<sup>13</sup> C <sub>22</sub> H <sub>22</sub> N <sub>2</sub> O <sub>4</sub>	379.1612	379.1629 (M + 1)*
C <sub>22</sub> H <sub>22</sub> N <sub>2</sub> O <sub>4</sub>	378.1579	378.1584 (M)*
C <sub>21</sub> H <sub>19</sub> N <sub>2</sub> O <sub>3</sub>	347.1394	347.1376
C <sub>21</sub> H <sub>18</sub> N <sub>2</sub> O <sub>3</sub>	346.1316	346.1350
C <sub>21</sub> H <sub>18</sub> N <sub>2</sub> O <sub>2</sub>	330.1368	330.1376
C <sub>22</sub> H <sub>16</sub> N <sub>2</sub> O	324.1261	324.1229
C <sub>20</sub> H <sub>17</sub> N <sub>2</sub> O <sub>2</sub>	317.1290	317.1286
C <sub>21</sub> H <sub>14</sub> N <sub>2</sub> O	310.1105	310.1091
C <sub>20</sub> H <sub>15</sub> N <sub>2</sub> O	299.1183	299.1219
C <sub>19</sub> H <sub>15</sub> N <sub>2</sub> O	287.1184	287.1186
C <sub>20</sub> H <sub>13</sub> N <sub>2</sub>	281.1077	281.1082
C <sub>20</sub> H <sub>12</sub> N <sub>2</sub>	280.0999	280.1005

Toxicity studies of alkaloids A-1, A-2, and B in chicken embryos (Table IV) indicate that *B. epichloë* can produce mycotoxins in vitro. This finding supports prior convictions (Bacon et al., 1975; Porter et al., 1974, 1975) that the fungus *Balansia* can produce indole alkaloids and thus may be involved in toxic syndromes observed in cattle grazed on parasitized grasses. Quantitatively (micrograms/egg), it appears that the potencies of the alkaloids tested ranged in the order of B > A2 ≈ A1 (Table IV). Limited testing (due to small quantities of alkaloids) of chicken embryos suggested that synergism may be involved in the potencies of these compounds. A solution of A-1, A-2, and B at 28, 10, and 11 μg, respectively, resulted in 100% mortality in 10 eggs. Alkaloids A-2 plus B at 6–7 μg each also resulted in 100% mortality (5 eggs). At 4–5 μg each, A-2 plus B resulted in 40% mortality (2 out of 5 eggs). The toxicity observed for the solution of these alkaloids was probably due to the synergism of A-2 and

Table IV. Toxicity of *B. epichloë* Alkaloids to Chicken Embryo

Alkaloid	Dosage, μg/egg	% mortality
A-1	57	53
	113	100
A-2	20	20
	60	55
	99	100
B	23	80
	68	100

B. It is unknown at present what effects the minor compound in B (e.g., C<sub>22</sub>H<sub>22</sub>N<sub>2</sub>O<sub>4</sub>, M<sup>+</sup> 378) contributes to the potency of this fraction.

The 3-indoleglycerol (B, M<sup>+</sup> 207) has been isolated from tryptophan requiring mutants of *Escherichia coli*, *Salmonella typhimurium* (Lingens et al., 1957), and also from *Neurospora crassa* (Lingens and Guck, 1963; Garrick et al., 1964); correspondingly blocked mutants of *Saccharomyces cerevisiae* accumulate 3-(3,3-diindolyl)propane-1,2-diol (A-2, M<sup>+</sup> 306) (Lingens and Goebel, 1967). It has been suggested (Preobrazhenkaya et al., 1969) that the 3-indoleglycerol (M<sup>+</sup> 207) may exist as the erythro-threo isomeric mixture under biosynthetic conditions. Conformational analyses of the natural products reported here are under investigation in order to define this possibility with *Balansia epichloë*.

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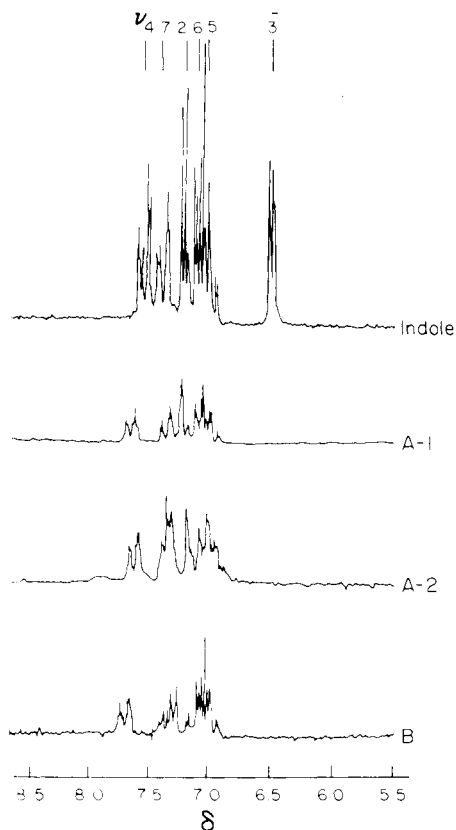


Figure 3. Pulsed NMR spectra ( $\text{CD}_3\text{OD}$ ) of *Balansia* alkaloids A-1, A-2, and B as compared with indole.

study.

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