Chemical Ionization Mass Spectrometry of Ergot Cyclol Alkaloids

James K. Porter* and Don Betowski[1]

The isobutane chemical ionization (CI) mass spectra of the ergot cyclol alkaloids are reported. The ergotamine, ergoxine, and ergotoxine groups are compared relative to the substituents attached to the tricyclic peptide portion of the alkaloids. Observations suggest that the major fragmentation pathway occurs by pyrolytic cleavage of the bond joining the tricyclic peptide moiety with the lysergic acid amide portion of the molecule. The utilization of isobutane CI is demonstrated as a complimentary technique to electron-impact (EI) mass spectra for differentiating these ergot cyclol alkaloids.

The ergot alkaloids produced by the fungus *Claviceps purpurea* (Clavicipitaceae) have been associated with a variety of toxic syndromes ("ergotism") in domestic animals (cattle, horses, sheep, and pigs) fed or grazed on grains or pasture grasses infected with this fungus. *C. purpurea* infects wheat, barley, rye, oats, and grasses such as wheat grass, quack grass, smooth bromegrass, wild ryegrass, and bluegrasses (Burfening, 1973). Of more than 600 recorded plants in Gramineae, over 400 are parasitized by *C. purpurea* (Bové, 1970). The variety of toxic syndromes exhibited by animals fed grain products infected or contaminated with *Claviceps* are lowered reproductive efficiency due to animals failure to cycle, failure to conceive, and/or failure to maintain pregnancy. Other syndromes observed are agalactia, lowered growth efficiency through decreased weight gains, elevated temperatures, increased respiration rates, lameness, convulsions, and death (Burfening, 1973). Contaminated feeds have a severe economic impact on both grain utilization and livestock production due to the variety of commodity grains and pasture grasses infected by this fungus. It is unknown what economic impact of chronic low level (subclinical) concentrations these compounds have on livestock production.

The alkaloids produced by *C. purpurea* may be separated into the clavine alkaloids, the lysergic acids, the simple lysergic acid amides, and the ergopeptines or cyclol alkaloids (Berde and Schild, 1978). The most pharmacologic active, naturally occurring, and generally considered most important class of these alkaloids is the ergopeptines or cyclols (Berde and Schild, 1978). Currently this class consists of twelve compounds (Table I), only two of which have not been found in nature (i.e., β-ergosine and β-ergoptine). These twelve alkaloids, based on the amino acids comprising the tricyclic peptide portion of the compound, are subdivided into three groups (Table I): (1) ergotamine, (2) ergoxine, and (3) ergotoxine groups (Brunner et al., 1979; Stadler et al., 1977). Previous reports (Floss, 1976) have shown production of these cyclol alkaloids were limited to the genus *Claviceps*. However, recent investigations involving "ergot-like" toxicities in cattle grazing on tall fescue (Bacon et al., 1977) has extended the cyclol alkaloid producing capabilities into another genus of the Clavicipitaceae, *Epichloë typhina* (Porter et al., 1979). *E. typhina*, unlike the localized seed head infections of *Claviceps*, is a systemic endophyte with the potential of causing great economic impact on livestock production. *E. typhina* has been reported on a number of grasses in North America (Kohlmeyer and Kohlmeyer, 1974; Sprague, 1950), including five species of *Festuca* (Sprague, 1950) and, more recently (Bacon et al., 1977), in tall fescue.

The similar structures and physical, chemical, and pharmacologic properties of these ergot cyclol alkaloids (Berde and Schild, 1978; Brunner et al., 1979; Floss, 1976; Stadler et al., 1977) prompted further investigations into identification techniques for this important agriculture and medicinal class of compounds.

Electron impact (EI) mass spectral studies of the ergot clavine and peptide (cyclol) alkaloids have been reported (Barber et al., 1965; Inoue et al., 1972; Schmidt et al., 1978; Voight et al., 1974; Vokoun et al., 1974; Vokoun and Rehacek, 1975). Interpretation of the cyclol alkaloid's EI mass spectra is complicated by absence of the molecular ion, the abundance of the diagnostic fragments for the tricyclic peptide portion of the molecule, and the almost identical chemical and physical properties of the more recently isolated natural ergopeptides (Brunner et al., 1979; Porter et al., 1979). In addition, the similarities of the UV, IR, and EI spectra among the homologous series of cyclols further complicates interpretation of these alkaloid mixtures. However, we have found the combination of chemical ionization (CI) (Arsenault, 1972; Fales et al., 1970) and EI mass spectrometry promising for the identification of these compounds. The interpretation of the CI spectra is based on the substituent differences (Table I, R_1 and R_2) in the tricyclic peptide (Figure 1) or cyclol portion of the alkaloid.

EXPERIMENTAL SECTION

Mass spectrometry–chemical ionization mass spectra were obtained by use of a Varian Mat 44 quadrupole mass spectrometer equipped with an EI/CI source. The samples were placed in the mass spectrometer with a direct inlet probe, and two different temperature programs were used: (1) increase from 80 to 250 °C, at 25 °C/min; (2) isothermal at 200 °C. The ion source was maintained at 200 °C and was operated at pressures of 170–300 μbar. The ion source voltages were adjusted to optimize the peaks for m/e 219, m/e 264, and m/e 414 with (perfluorotributyl)amine as a calibration compound. The electron voltage was kept in the range 120–200 V. Isobutane was the reactant gas in all cases. The three major ions characteristic of the three major alkaloid fragments are listed in Table I as relative intensities based on total ionization of the compounds. As examples, only those ions occurring at ≥2% of the base peak are listed in Figures 2 and 3.

The following alkaloids were obtained from Dr. P. A. Stadler, Sandoz, Basel, Switzerland: ergosine, β-ergosine, ergovaline, ergostine, ergoptine, β-ergoptine, ergonine, and α- and β-ergokryptine. Ergotamine, ergocristine, and ergocornine were obtained from Sigma Chemical Co. All compounds were checked for purity by TLC prior to use

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Table I. Isobutane Chemical Ionization of Ergot Peptide Alkaloids

	M_r	R_2	A′	B′	C′
ergotamine group (R_1 = CH_3)					
ergotamine	581	ϕCH_2	268 (72)	315 (100)	245 (17)
ergosine	547	*i*-Bu	268 (70)	281 (100)	211 (17)
β-ergosine	547	*sec*-Bu	268 (100)	281 (37)	211 (6)
ergovaline	533	*i*-Pr	268 (40)	267 (81)	197 (100)
ergoxine group (R_1 = C_2H_5)					
ergostine	595	ϕCH_2	268 (74)	329 (100)	245 (37)
ergoptine	561	*i*-Bu	268 (100)	295 (68)	211 (6)
β-ergoptine	561	*sec*-Bu	268 (100)	295 (18)	211 (2)
ergonine	547	*i*-Pr	268 (100)	281 (42)	197 (4)
ergotoxine group (R_1 = *i*-Pr)					
ergocristine	609	ϕCH_2	268 (100)	343 (55)	245 (47)
α-ergokryptine	575	*i*-Bu	268 (100)	309 (30)	211 (3)
β-ergokryptine	575	*sec*-Bu	268 (100)	309 (53)	211 (4)
ergocornine	561	*i*-Pr	268 (100)	295 (92)	197 (63)

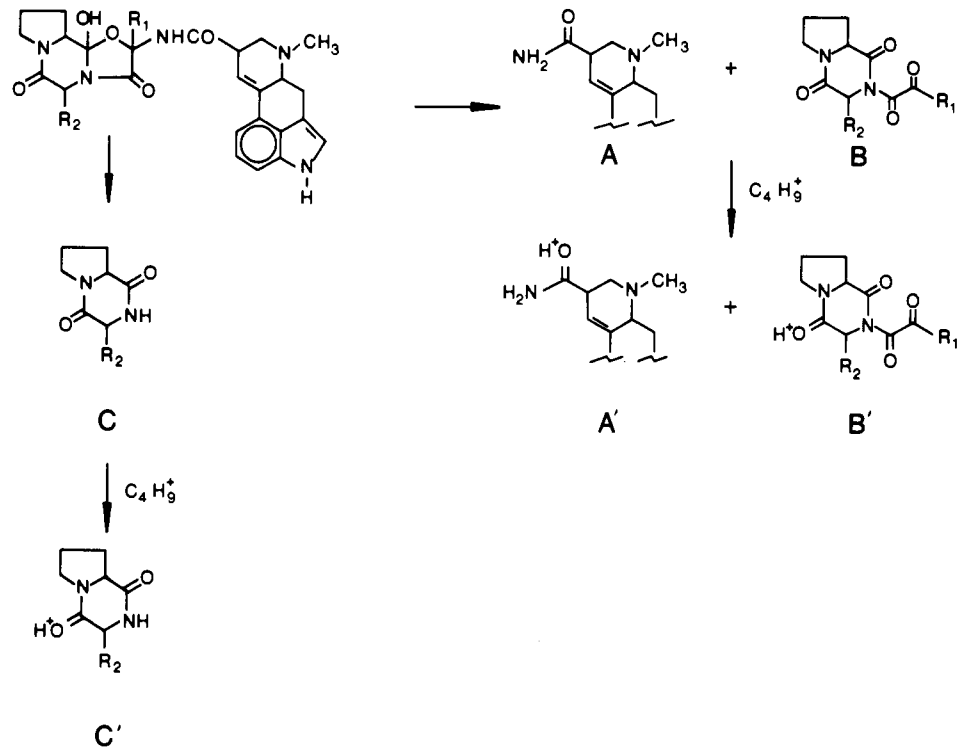

Figure 1. Isobutane chemical ionization of ergot peptide alkaloids.

and shown to be a single entity in several solvent systems (Brunner et al., 1979; Porter et al., 1979) when visually observed under a 254- and 366-nm light source and after the plates had been sprayed with *p*-(dimethylamino)-benzaldehyde.

RESULTS AND DISCUSSION

The major ions characteristic of the isobutane chemical ionization spectra for the ergotamine, ergoxine, and ergotoxine alkaloids are reported in Table I. These results suggest that major fragments for the cyclol alkaloids are due to pyrolytic cleavage of the bond joining the tricyclic peptide moiety (Figure 1) with the lysergic acid amide portion of the molecule. Also, it is suggested that proton transfer from the cyclol moiety occurs prior to ion–molecule reaction. Even under the conditions of CI reported, the $(M + 1)^+$ for these compounds was not observed. However, the ions A′, B′, and C′ were observed, presumably due to lysergic acid amide (A) and the diketopiperazines (B and C), respectively (Figure 1). These fragments correspond to the ions A^+, B^+, and C^+ (Vokoun et al., 1974; Vokoun and Rehacek, 1975) in the EI spectrum. The diketopiperazines B and C are referred to as the pyroergopeptines (Stadler, 1979). Thus, pyrolysis of these alkaloids yields three neutral fragments which then undergo ion–molecule reaction with isobutane and results in the abundant fragments characteristic of the lysergic acid amide and the cyclol portions of the compounds. These fragments are indicative of the substituents at R_1 and R_2. Ergotamine (Table I; Figure 2) yields the peptide fragment B [EI, *m/e* 314 (Figure 1)], which under CI is protonated and yields *m/e* 315 [100% (Figure 1, B′)]. The neutral diketopiperazine C may be formed via B by proton transfer from R_1 with loss of CH_2CO and CO, respectively. Also, C may occur directly from the cyclol portion of the alkaloid by proton transfer from OH with concurrent loss of R_1CO-lysergamide and CO (Figure 1). This fragment would then produce C′ [*m/e* 245, 17% (Table I)] on reaction with isobutane. The lysergic acid amide fragment [EI, *m/e* 267 (Figure 1, A)] undergoes the same reaction and thereby results in *m/e* 268 [72% (Table I, A′)]. The chemical ionization mass spectrum of ergocristine (Figure 3) also is given as an example.

Subsequent to the above, the ionization of fragments A′, B′, and C′ (Figure 1) was monitored as a function of temperature (i.e., programmed at 25 °C/min to 250 °C and

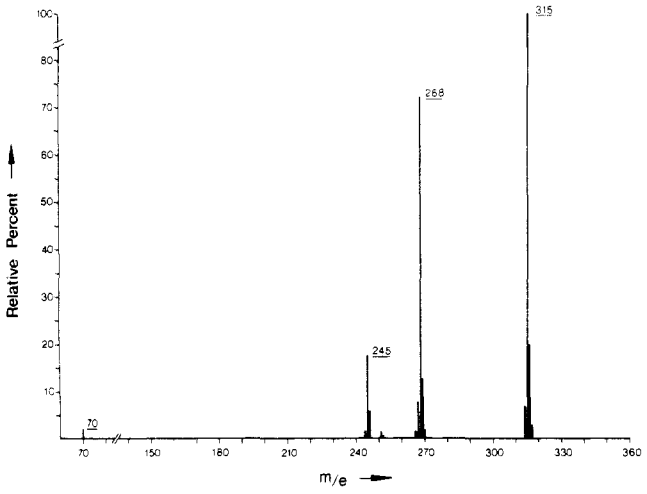

Figure 2. Chemical ionization mass spectrum of ergotamine.

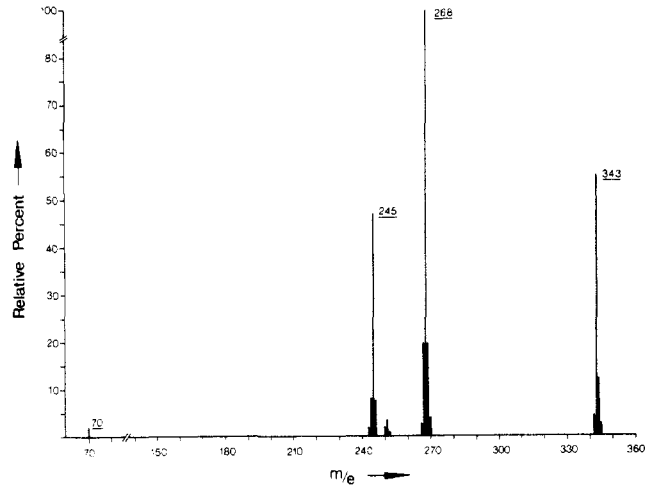

Figure 3. Chemical ionization mass spectrum of ergocristine.

also isothermal at 200 °C) and plotted relative to the total ionization of the compounds (Figures 4 and 5). The appearance of the *m/e* 245 fragment's maximum intensity just after *m/e* 315 (Figure 4, ergotamine) and *m/e* 343 (Figure 5, ergocristine) may indicate formation of C dependent on B. However, recent studies using ^{13}C-labeled ergostine (Belzecki et al., 1980) containing two deuterium atoms at R_1 (i.e., $-CD_2CH_3$) have shown that a major P + 3 isotope occurred for this fragment, while fragment *m/e* 245 remained unchanged. This would seem to support the latter and not the former of the mechanisms mentioned above in the formation of these fragments. Also, this may be just a reflection of the thermal stabilities of A, B, and C and thus suggests that the relative ion intensities observed for these compounds depend on instrument temperature. That thermal decomposition precedes ion–molecule reaction is also supported by evidence of ions occurring due to the condensation reactions of A, B, and C with $C_4H_9^+$ and $C_3H_7^+$, respectively (≤2%). Also, evidence was observed for the reaction of B + B′ and B + C′ (or B′ + C). As observed above for the major diagnostic ions, the incidences of these condensation reactions, as indicated by the relative intensities of the ions formed depend on compound quantity in the vapor phase and instrument pressure and temperature at which pyrolysis and fragmentation occur.

When $R_2 = \phi CH_2$ (i.e., ergotamine, ergostine, and ergocristine), EI (i.e., 70 eV) yields the peptide fragments (≥5%) corresponding to B^+ and C^+ (Figure 1) as ions which are easily distinguished (Vokoun and Rehacek,

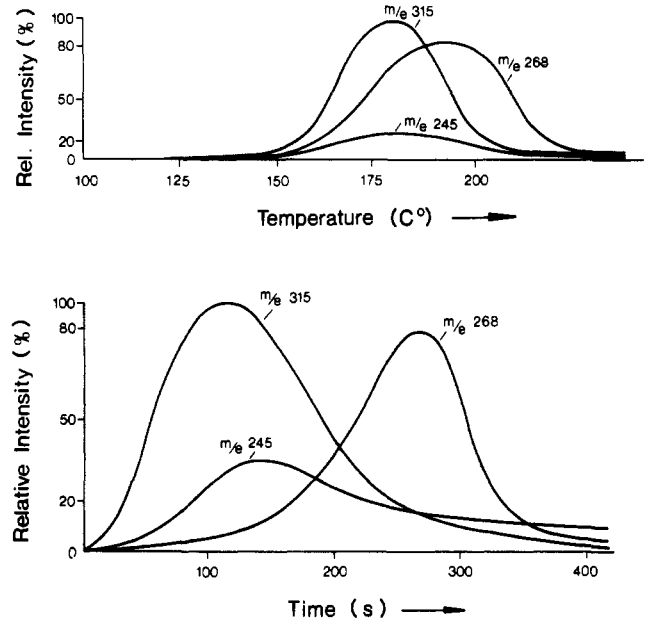

Figure 4. Chemical ionization of ergotamine programmed at 25 °C/min (upper) and isothermal at 200 °C (lower).

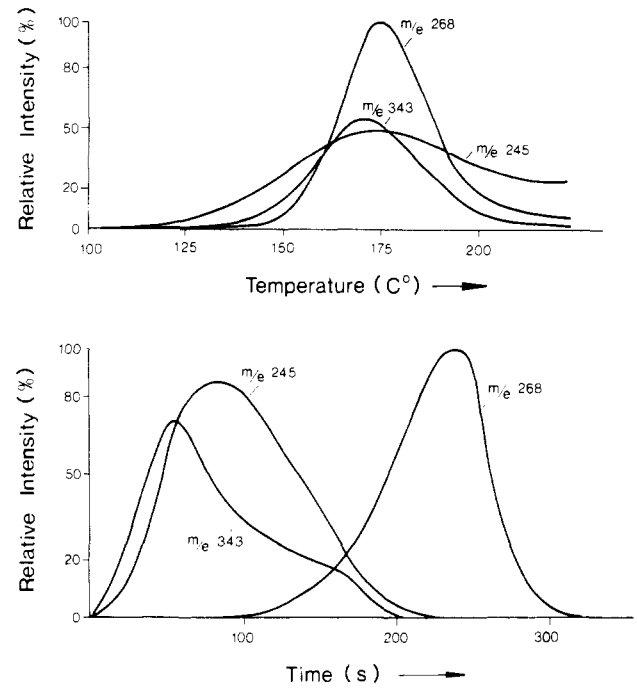

Figure 5. Chemical ionization of ergocristine programmed at 25 °C/min (upper) and isothermal at 200 °C (lower).

1975). Also, fragment *m/e* 91 is one of the major ions (≥40%) in these spectra. However, when R_2 = alkyl (i.e., isobutyl, *sec*-butyl, and isopropyl), the diagnostic peptide fragments B^+ and C^+ are somewhat nebulous (≤1%) (Vokoun et al., 1974; Vokoun and Rehacek, 1975). Also, for ergovaline the fragments corresponding to *m/e* 266 and *m/e* 196 (EI, B^+, and C^+, Figure 1) occur at that portion of the spectrum isobaric with the fragment ions from lysergamide. Thus, from the fragment ions B′ and C′ (Table I), respectively, CI may be used in distinguishing the moieties attached at R_1 and R_2 (Figure 1). For ergosine (Table I) fragments B′ (*m/e* 281, 100%) and C′ (*m/e* 211, 17%) are supportive of $R_1 = CH_3$, and $R_2 = C_4H_9$. For ergoptine B′ (*m/e* 295, 68%) and C′ (*m/e* 211, 6%) are supportive of $R_1 = C_2H_5$ and $R_2 = C_4H_9$. Ergocornine yields *m/e* 295 (B′) at 92%, and *m/e* 197 (C′) at 63%

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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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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