

Fragmentation patterns of selected ergot alkaloids by electrospray ionization tandem quadrupole mass spectrometry

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Tall fescue toxicosis and other maladies in livestock result from the ingestion of vasoconstrictive ergot alkaloids produced by fungal endophytes associated symbiotically with the grass. In order to facilitate future analyses of grass extracts considered responsible for outbreak of related livestock diseases, we examined the electrospray ionization mass spectra of specific ergot alkaloids under conditions that permit protonation. Our purposes were both to record the spectra with interpretation of mechanisms of fragmentation and to derive commonalities that would allow the prediction of mass spectra of related compounds for which standards were not readily available. With $[M + H]^+$ values in parentheses, water-insoluble lysergic acid peptide ergot derivatives ergovaline (m/z 534), ergotamine (m/z 582), ergocornine (m/z 562), ergocryptine (m/z 576) and ergocrystine (m/z 610) exhibited a consistent loss of water (-18 u) from the C-12' α -hydroxy functionality. Of this group, ergovaline and ergotamine generated an m/z 320 fragment deriving from cleavage of ring E amide and ether functions with retention of the peptide ring system methyl group. Ergocornine, ergocryptine and ergocrystine similarly formed an m/z 348 fragment with retention of isopropyl. These assignments were supported by the lack of similar fragments from the water-soluble ergot ergonovine, which lacks a peptide ring system. Clavine-type ergot alkaloids lysergic acid and lysergol lack any substituents beyond simple ones directly on the C-8 position and, similarly to ergonovine, lack significant fragments at m/z 268, 251 and 225 shared by the peptide ergot alkaloids. Copyright © 2004 John Wiley & Sons, Ltd.

KEYWORDS: ergot alkaloids; tall fescue toxicosis; mare reproductive loss syndrome

INTRODUCTION

Tall fescue toxicosis in livestock appears to result from ingestion of grasses harboring a complex symbiotic relationship involving fungal endophyte habitation. The perennial grass *Festuca arundinacea*, or tall fescue, spreads via seed propagation on over 35 million acres of pasture in the USA.^{1,2} Nutritionally it is considered one of the best forages for livestock, including horses.³ Since it is one of the most widely known forage grasses for horses, its toxic properties have recently been considered of possible significance in

equine reproductive processes⁴ and also in the April–May 2001 outbreak of mare reproductive loss syndrome (MRLS) in Kentucky Bluegrass horse farms⁵ (reports and updates on this syndrome are available on the Internet⁶). This is of particular interest owing to previous studies describing vasoconstrictive and uterine-stimulating properties of certain endophyte alkaloids.^{1,4,7,8}

While it is known that different endophytic fungi infect different species of grasses and produce different arrays of toxins,⁹ one pharmacologically important group of indole alkaloids derived from seedhead-infecting fungi are the ergoline or ergot-type alkaloids; these are isolated from the dried sclerotium of the fungus *Claviceps purpurea*, a parasite on many grasses, rye, wheat and other grains.¹⁰ There are three main groups of such ergot alkaloids, the clavine group, the water-soluble lysergic acid type and the water-insoluble lysergic acid type or peptide ergot alkaloids (see general structure, Fig. 1). These alkaloids are not especially useful pharmacologically, but agroclavine is a powerful uterine stimulant and many of the ergot alkaloids are prolactin-release inhibitors. Ergonovine has potent

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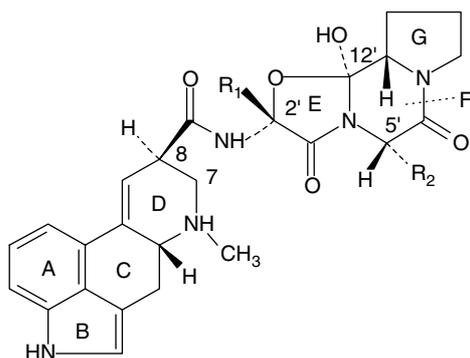


Figure 1. General structure of peptide ergot alkaloids indicating stereochemistry and important numeric labels of positions and alphabetical labeling of rings. Where R_1 = methyl, R_2 is the following in the compounds in parentheses: isopropyl (ergovaline); benzyl (ergotamine). Where R_1 = isopropyl, R_2 is the following in the compounds in parentheses: isopropyl (ergocornine); isobutyl (ergocryptine); benzyl (ergocryptine). Structure and labels after Rall and Schleifer¹¹ and Petricková *et al.*²⁰

uterine contraction activity and is used in treating postpartum hemorrhages. It also has significant vasoconstrictor activity.¹¹

There is a distinct need for understanding the fragmentation mechanisms of these classes of toxins as revealed by the relatively newer ionization methods of atmospheric pressure ionization (API) combined with mass spectrometry (MS). The impetus for such research was the discovery of unknown endophyte-associated alkaloid peaks on high-performance liquid chromatography (HPLC) traces of clinical sample extracts involving suspected fescue toxicosis from Oregon State University's Diagnostic Laboratory in Oregon. In order to identify new and possibly toxic ergopeptides and related unknown chromatographic peaks, characterization of known standards and their metabolic products was essential. Several standards of ergot alkaloids were examined, including clavine-type (lysergic acid, lysergol), water-soluble lysergic acid derivatives (ergonovine) and water-insoluble lysergic acid peptide derivatives (ergotamine, ergocornine, ergocryptine, ergovaline, ergocryptine) for patterns of positive electrospray ionization (ESI(+))MS fragmentation. The resultant understanding of fragmentation events facilitated HPLC/MS/MS examination of methanol extracts of grasses for ergot alkaloids, as will be reported separately. This work builds on earlier work by Yates *et al.*,¹² who carried out similar analytical method development, but used isobutane negative ion chemical ionization (NICI) of thermally desorbed and partially fragmented compounds, and on work of Shelby and Flieger, who developed gradient HPLC/fluorescence methods with LC/MS identification of over 10 ergots following epimerization.¹³ The soft ionization techniques of electrospray utilized in our current approach provide the advantage of avoiding the preliminary fragmentation of NICI-MS in data reported here. Chromatographic work extending that of Shelby and Flieger will be reported in a future paper.

EXPERIMENTAL

Compounds

Ergovaline was isolated from tall fescue as described previously.¹⁴ Its concentration was estimated spectrophotometrically and diluted to $1.0 \mu\text{g ml}^{-1}$ in methanol. Other ergot alkaloids were obtained from Sigma. Ergonovine, ergotamine tartrate, ergocryptine, ergocryptine, lysergic acid and lysergol standards were prepared as $1.00 \mu\text{g ml}^{-1}$ stock solutions of the parent compound in methanol. Ergocornine was prepared similarly but was more readily soluble in ethyl acetate than in methanol.

Electrospray ionization mass spectrometry

Ergot alkaloid standards or extracts were prepared for direct infusion ESI(+)-MS analysis by dilution 1:5 or 1:10 with 0.05% aqueous formic acid-acetonitrile (1:1). Infusion was carried out with a Harvard syringe pump equipped with a 500 μl Hamilton gas-tight syringe with infusion at 1.0 ml h^{-1} . The mass spectrometer was a Micromass (Beverly, MA, USA) Quattro II ESI-MS/MS, and typical ESI-MS voltage settings for detection and analysis of various ergots were as follows: capillary, 3.15 kV; HV lens, 0.5 kV; cone, 30 V; skimmer lens, 1.0 V; r.f. lens, 0.1 V; source temperature, 120°C ; argon pressure for collisionally induced dissociation (CID) experiments, $(3-4) \times 10^{-3}$ mbar; and ionization energies, MS1 0.5 V and MS2 13.0 V. The collision energy was set between 24 and 32 eV. ESI mass and tandem mass spectra were acquired as continuum data for a minimum of 1–2 min over the m/z 10–800 range, applying 1.8 s per scan duration. Resultant data were background subtracted and smoothed with Micromass MassLynx version 3.4 software. Spectra were deconvoluted with the assistance of Mass Spec Calculator Pro software, version 4.03 (Quadtech Associates, 1998). Where appropriate, optimized semi-empirical three-dimensional structures were computed by application of an AM1 subprogram of HyperChem, Release 3 (Autodesk, 1993). Mass spectral fragmentation schemes were derived with reference to McLafferty and Turecek.¹⁵

RESULTS

Ergovaline

Ergovaline combines a methyl group on its five-membered lactam ring E with an isopropyl group on its peptide ring system central F ring (for ring designations, see Fig. 1). Figure 2 shows the ergovaline and fragment ion mass spectra (A and B, respectively), with interpretations of fragments ions. Cleavages to release the m/z 223 fragment and its demethylated counterpart at m/z 208 are illustrated in Fig. 2B (inset). Cleavage at the carbonyl group of the amide functionality is expected to generate an even-electron cation, designated EE^+ , at m/z 223;¹⁵ therefore, assuming a two-stage fragmentation, loss of CH_3 to generate m/z 208 must proceed by elimination of CH_3^\bullet as a radical and would thus represent a violation of the even-electron rule,¹⁶ although many exceptions to this rule are known to exist.^{16–19} This rule can be simply summarized as follows: $EE^+ \rightarrow EE^0[\text{neutral}] + EE^+$ fragmentations are normally greatly favored over

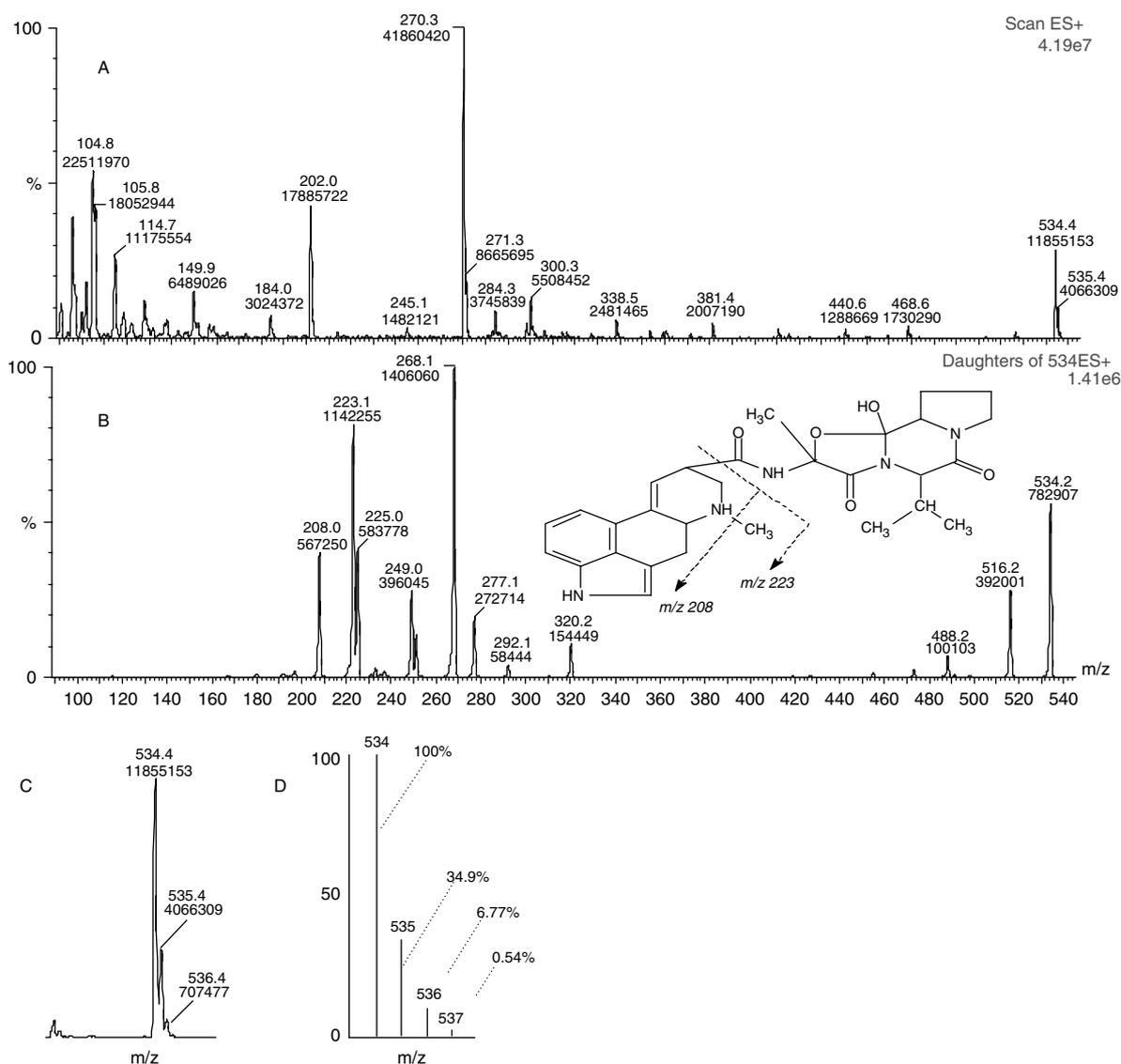


Figure 2. Ergovaline mass spectrometry. (A) ESI(+) mass spectrum of ergovaline standard infused as a $0.2 \mu\text{g ml}^{-1}$ solution in acetonitrile–0.05% formic acid at 1.0 ml h^{-1} into the Quattro II mass spectrometer. (B) Fragment ion spectrum of the m/z 534.4 fragment seen in (A). Fragment ion principal peak assignments: m/z 534, $[\text{M} + \text{H}]^+$; 516, loss of water (-18) from peptide ring system; 488, m/z 516 minus CO; 320, cleavage of peptide ring system (in amide and at ether); 292, m/z 516 minus lysergic ring system; 277, m/z 292 minus CH_3 ; 268, [lysergic ring system- $\text{C}=\text{O}-\text{NH}_2$] + H; 249, m/z 516 minus [lysergic ring system- $\text{C}=\text{O}-\text{NH}_2$]; 225, probably a $+\text{H}_2$ variant of m/z 223; 223, $[\text{M} + \text{H}]^+$ minus [peptide side chain- $\text{NH}-\text{HC}=\text{O}$]; 208, m/z 223 minus CH_3 ; 197, m/z 223 minus $\text{CH}\equiv\text{CH}$; 180, m/z 223 minus $\text{CH}_2=\text{NCH}_3$; 70, pyrrole ring + H; 44, $\text{CH}_2=\text{NHCH}_3$. The inset shows the ergovaline structure protonated on the lysergic ring tertiary amine and indicating CID with the origins of the m/z 223 and 208 fragments. Peak labels in this and subsequent spectra show measured m/z value (upper value) and measured intensity (lower value). (C) Measured signal intensities in the vicinity of the $[\text{M} + \text{H}]^+$ molecular ion. (D) calculated signal intensities (MS Calculator Pro).

$\text{EE}^+ \rightarrow \text{OE}^+ + \text{OE}^{+\bullet}$, where OE represents an odd-electron species. The apparent m/z 223 \rightarrow 208 process became a recurring fragmentation motif with these compounds, and is examined with the next compound ergotamine in greater detail.

Figure 2(C) expands measured abundances, in the vicinity of the $[\text{M} + \text{H}]$ pseudomolecular ion of ergovaline, m/z 534.4, with abundance values for $[\text{M} + 1 + \text{H}]$ at 535.4, and $[\text{M} + 2 + \text{H}]$ at 536.4 of 34.3 and 6.0%, respectively, relative to $[\text{M} + \text{H}]$. These correspond well with those calculated by Mass Spec Calculator software for a $\text{C}_{29}\text{H}_{36}\text{N}_5\text{O}_5$ compound, as illustrated in Fig. 2(D). Note the significant m/z 270 peak

found in the ESI(+)-MS scan in Fig. 2(A), which was found to be a recurring contaminant in other preparations.

Ergotamine

Ergotamine combines a methyl group on its five-membered lactam ring with a benzyl group on its peptide ring system central ring. As seen with ergovaline in Fig. 2(B), the recurring tertiary alcohol again favored CID-induced dehydration, and the ergotamine and fragment ion mass spectra are shown in Fig. 3(A) and (B). Ergotamine $[\text{M} + \text{H}]$, $[\text{M} + 1 + \text{H}]$ and $[\text{M} + 2 + \text{H}]$ abundances were 100, 39.8 and 8.8%, respectively, which compared well with values of

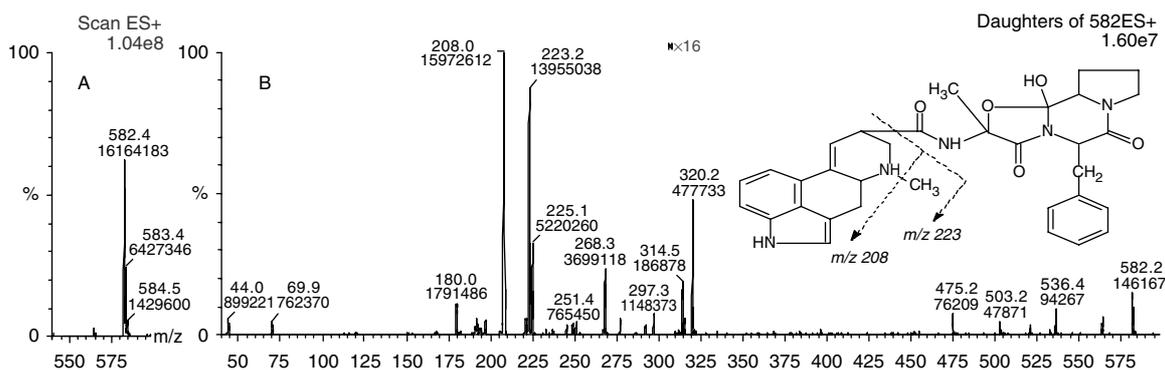


Figure 3. Ergotamine mass spectrometry. (A) ESI(+) mass spectrum of ergotamine infused as a $0.2 \mu\text{g ml}^{-1}$ solution in acetonitrile–0.05% formic acid at 1.0 ml h^{-1} into the Quattro II mass spectrometer. (B) Fragment ion spectrum of the m/z 582.4 fragment seen in (A); note the 16-fold enhancement of fragment signal intensities above m/z 310. Fragment ion principal peak assignments: m/z 582, $[M + H]^+$; 564, loss of water (-18) from peptide ring system; 536, m/z 564 minus CO; 503, $[M + H]^+$ minus C_6H_5 with transfer of a proton; 475, $[M + H]^+$ minus CH_3 and $\text{CH}_2\text{C}_6\text{H}_5$ groups with transfer of a proton; 320, cleavage of peptide ring system (in amide and at ether); 297, 564 minus [lysergic ring system- CONH_2]; 268, [lysergic ring system- $\text{C=O}-\text{NH}_2$] + H; 225, probably a $+H_2$ variant of m/z 223; 223, $[M + H]^+$ minus [peptide side-chain- $\text{NH}-\text{HC=O}$]; 208, m/z 223 minus CH_3 ; 197, m/z 223 minus $\text{CH}\equiv\text{CH}$; 180, m/z 223 minus $\text{CH}_2=\text{NCH}_3$; 70, pyrrole ring + H; 44, $\text{CH}_2=\text{NHCH}_3$. The inset shows the ergotamine structure protonated on the lysergic ring tertiary amine and indicating CID with the origins of the m/z 223 and 208 fragments.

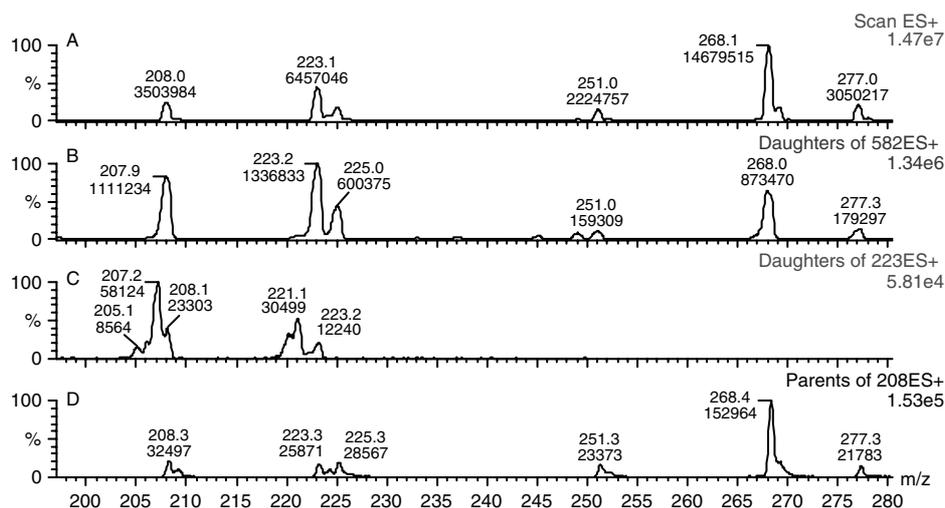


Figure 4. Relevant spectra demonstrating violation of the even-electron rule in ergotamine. A $10 \mu\text{g ml}^{-1}$ solution of ergotamine tartrate in 0.5% formic acid–acetonitrile (1 : 1) was infused into the Quattro II ESI(+) mass spectrometer at 1.2 ml h^{-1} , with a relatively high cone voltage setting of 50 V; the collision energy was 24 eV where appropriate. Scans from top to bottom: (A) full scan; (B) m/z 582 fragment ion scan; (C) m/z 223 fragment ion scan; (D) m/z 208 parent ion scan. ACD/MS Fragmenter software, version 1.01 (2003; ACD Laboratories, Toronto, Canada) indicates likely origins for the m/z 223 fragments in (C) as m/z 221, loss of H_2 ; 208, loss of CH_3 ; 207, loss of CH_4 ; 206, loss of NH_3 ; 220 would involve unusual loss of H_3 , from which loss of CH_3 would then account for 205.

100, 39.3 and 8.4% for a $\text{C}_{33}\text{H}_{36}\text{N}_5\text{O}_5$ compound. Cleavages to release the m/z 223 fragment and its demethylated counterpart at m/z 208 are illustrated in Fig. 3B (inset). The caption lists peak assignments for the major ergotamine fragment ions.

As mentioned in the section on ergovaline, generation of m/z 223 and 208 by two-step fragmentation would involve processes summarized as $\text{EE}^+ \rightarrow \text{OE}^+ + \text{OE}^+$, generally considered to be disallowed owing to the requirement for electron pair separation. Since, as will be seen, these fragments occurred in all the ergots examined, except lysergol, we examined this phenomenon in greater depth. Figure 4(A)

displays a selected m/z 200–280 range of an ergotamine ESI(+) mass spectrum run as in Fig. 3 except with a high cone voltage of 50 V intended to induce in-source fragmentation; both m/z 223 and 208 became visible under these conditions. Figure 4(B) demonstrates that $[M + H]$ fragment ions of m/z 582 were generally similar in pattern to those obtained in Fig. 3(B) under these conditions. Figure 4(C) shows that fragment ion analysis of the in-source generated m/z 223 fragment included m/z 208 among its progeny ions, demonstrating that this fragmentation is in fact an exception to the even-electron rule. However, the m/z 208 fragment was somewhat obscured by a significant m/z 207 ion as part of a

cluster of ions from m/z 205 to 208. Therefore, a parent ion scan for the m/z 208 product was included (Fig. 4(D)), which more clearly demonstrated its origin from m/z 223, and also from m/z 225, 251, 268 and 277, all ions likely to include the lysergic acid ring system (rings A–D in Fig. 1). The results in Fig. 4 were not peculiar to ergotamine, as nearly identical results were obtained from ergocryptine (not shown).

Ergocornine

Ergocornine, the ESI(+) mass spectrum of which is shown in Fig. 5(A), is the first of three compounds with an isopropyl group on its five-membered lactam E ring; it also includes an isopropyl group on its peptide ring system central ring. Ergocornine $[M + H]^+$, $[M + 1 + H]^+$ and $[M + 2 + H]^+$ abundances were 100, 34.5 and 5.0%, respectively, which compared relatively well with values of 100, 37.1 and 7.6%

for a $C_{29}H_{36}N_5O_5$ compound. This compound was capable of undergoing dehydration to an m/z 544.2 derivative, which was in turn responsible for several of the principal fragmentations, in particular at m/z 305, 277, 261 and 195 (Fig. 5(B)). Cleavages to release the m/z 223 fragment and its demethylated counterpart at m/z 208 are illustrated in Fig. 5B (inset). The caption summarizes fragment descriptions or cleavage events giving rise to each principal ion.

Ergocryptine

Ergocryptine differs from ergocornine only in the presence of an additional side-chain methylene group converting an isopropyl to an isobutyl group. It also underwent dehydration, and yielded other peaks similar to those of ergocornine (ergocryptine and fragment ion mass spectra are shown in Fig. 6(A) and (B), respectively). $[M + H]^+$, $[M +$

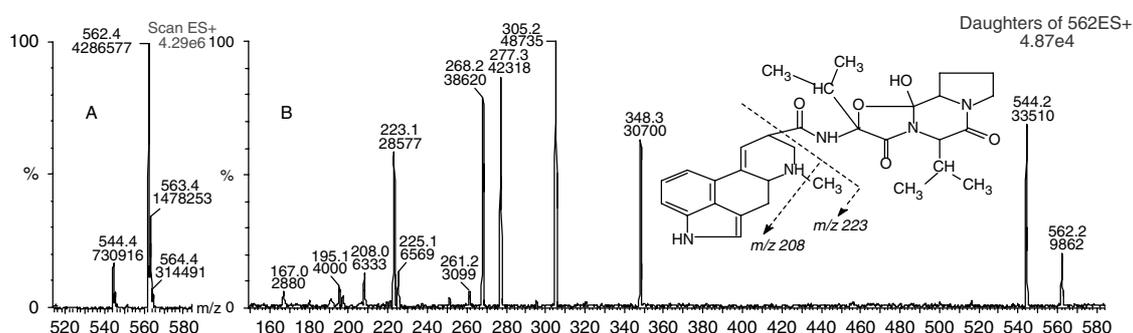


Figure 5. Ergocornine mass spectrometry. (A) ESI(+) mass spectrum of ergocornine infused as a $0.2 \mu\text{g ml}^{-1}$ solution in acetonitrile–0.05% formic acid at 1.0 ml h^{-1} into the Quattro II mass spectrometer. (B) Fragment ion spectrum of the m/z 562 fragment seen in (A). Fragment ion principal peak assignments: m/z 562, $[M + H]^+$; 544, loss of water (-18) within peptide ring system; 516 (minor), m/z 544 minus CO; 348, cleavage within and loss of most of peptide ring system (in amide and at ether); 305, m/z 544 minus R_2 group plus cleavage of lysergic D-ring to retain first two carbons; 277, m/z 544 minus [lysergic ring system–CONH₂]; 268, [lysergic ring system–C=O–NH₂] + H; 225, probably a $+H_2$ variant of m/z 223; 223, $[M + H]^+$ minus [peptide side-chain–NH–HC=O]; 208, m/z 223 minus CH₃; 197, m/z 223 minus CH≡CH; 180, m/z 223 minus CH₂=NCH₃; 167, m/z 223 minus CH₂=CHNCH₃; 70, peptide ring system pyrrole (C₄H₈N); 44, CH₂=NHCH₃. The inset shows ergocornine structure protonated on the lysergic ring tertiary amine and indicating CID with the origins of the m/z 223 and 208 fragments.

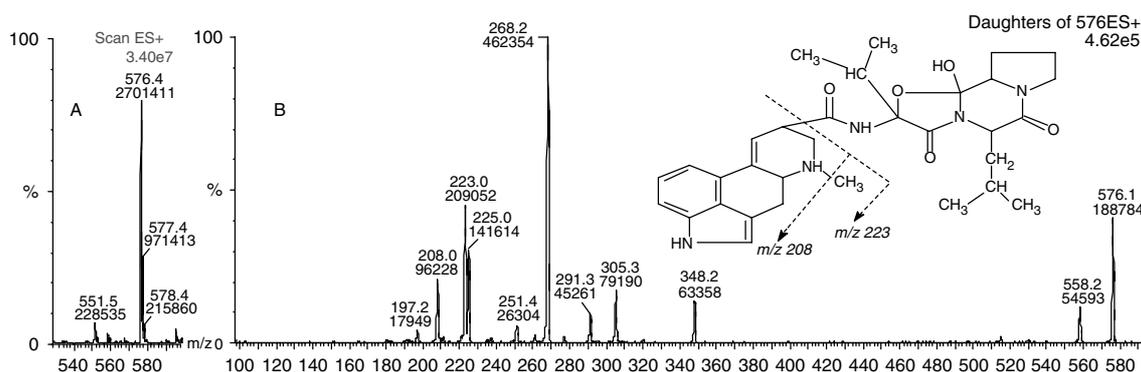


Figure 6. Ergocryptine mass spectrometry. (A) ESI(+) mass spectrum of α -ergocryptine infused as a $0.2 \mu\text{g ml}^{-1}$ solution in acetonitrile–0.05% formic acid at 1.0 ml h^{-1} into the Quattro II mass spectrometer. (B) Fragment ion spectrum of the m/z 576.4 fragment seen in (A). Fragment ion principal peak assignments: m/z 576, $[M + H]^+$; 558, loss of water (-18) from peptide ring system; 530 (minor), m/z 558 minus CO; 348, cleavage within and loss of peptide ring system (in amide and at ether); 305, m/z 558 minus R_2 group plus cleavage of lysergic D-ring to retain first two carbons; 291, m/z 558 minus [lysergic ring system–C=O–NH₂]; 268, [lysergic ring system–C=O–NH₂] + H; 225, probably a $+H_2$ variant of m/z 223; 223, $[M + H]^+$ minus [peptide side chain–NH₂–HC=O]; 208, m/z 223 minus CH₃; 197, m/z 223 minus CH≡CH; 180, m/z 223 minus CH₂=NCH₃; 70, pyrrole ring + H; 44, CH₂=NHCH₃. The inset shows ergocryptine structure protonated on the lysergic ring tertiary amine and indicating CID with the origins of the m/z 223 and 208 fragments.

1 + H] and [M + 2 + H] abundances were 100, 36.0 and 8.0%, respectively, which compared well with calculated values of 100, 38.3 and 8.0% for a C₃₂H₄₂N₅O₅ compound. Cleavages to release the *m/z* 223 fragment and its demethylated counterpart at *m/z* 208 are illustrated in Fig. 6B (inset). The differences in peak relative intensities from those of ergocornine may be a consequence of slight differences in tuning parameters. Peak assignments for principal peaks are given in the caption.

Ergocrystine

Ergocrystine has a benzyl group as in ergotamine, but also contains an isopropyl group on its five-membered lactam E ring as in ergocornine and ergocryptine. It also underwent dehydration, and yielded other peaks similar to those of ergocornine. Figure 7(A) and (B) show the ergocrystine and fragment ion mass spectra, respectively, and interpretations of fragment ions are given in the caption.

[M + H], [M + 1 + H] and [M + 2 + H] abundances were 100, 40.3 and 9.7%, respectively, which compared well with values of 100, 41.6 and 9.3% calculated for a C₃₅H₄₀N₅O₅ compound. Cleavages to release the *m/z* 223 fragment and its demethylated counterpart at *m/z* 208 are illustrated in Fig. 7B (inset).

Ergonovine

Ergonovine, the ESI(+) mass spectrum of which is shown in Fig. 8(A), and the fragment ion spectrum in Fig. 8(B), showed a pattern of *m/z* 223 and 208 fragment ions similar to those in ergovaline, ergotamine, ergocornine, ergocryptine and ergocrystine, which confirms the structural assignments for these fragments since ergonovine lacks the peptide ring system and must therefore derive *m/z* 223 and 208 from the lysergic ring system held in common. Likewise, the previous ergots displayed dehydration peaks generally in both ESI(+)-MS scans and fragment ion spectra, which phenomenon was

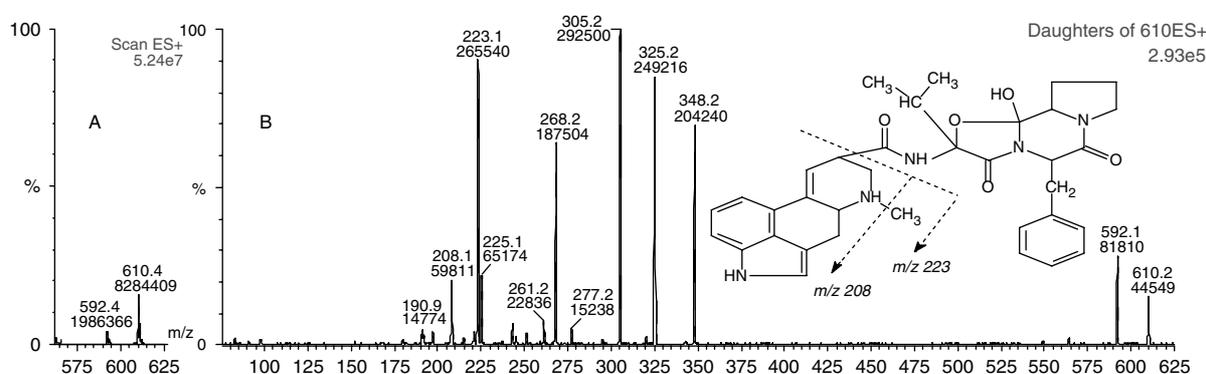


Figure 7. Ergocrystine mass spectrometry. (A) ESI(+) mass spectrum of ergocrystine standard infused as a 0.2 $\mu\text{g ml}^{-1}$ solution in acetonitrile–0.05% formic acid at 1.0 ml h^{-1} into the Quattro II mass spectrometer. (B) Fragment ion spectrum of the *m/z* 610.4 fragment seen in (A). Fragment ion principal peak assignments: *m/z* 610, [M + H]⁺; 592, loss of water (–18) from peptide ring system; 564 (minor), *m/z* 592 minus CO; 348, cleavage of peptide ring system (in amide and at ether); 325, *m/z* 592 minus [lysergic ring system–C=O–NH₂]; 305, *m/z* 592 minus R₂ group plus cleavage of lysergic D-ring to retain first two carbons; 268, [lysergic ring system–C=O–NH₂] + H; 225, probably a +H₂ variant of *m/z* 223; 223, [M + H]⁺ minus [peptide side-chain–NH₂–HC=O]; 208, *m/z* 223 minus CH₃; 197, *m/z* 223 minus CH≡CH; 191, *m/z* 325 minus isopropyl and benzyl; 180, 223 minus CH₂=NCH₃; 70, pyrrole ring + H; 44, CH₂=NHCH₃. The inset shows the ergocrystine structure protonated on the lysergic ring tertiary amine and indicating CID with the origins of the *m/z* 223 and 208 fragments.

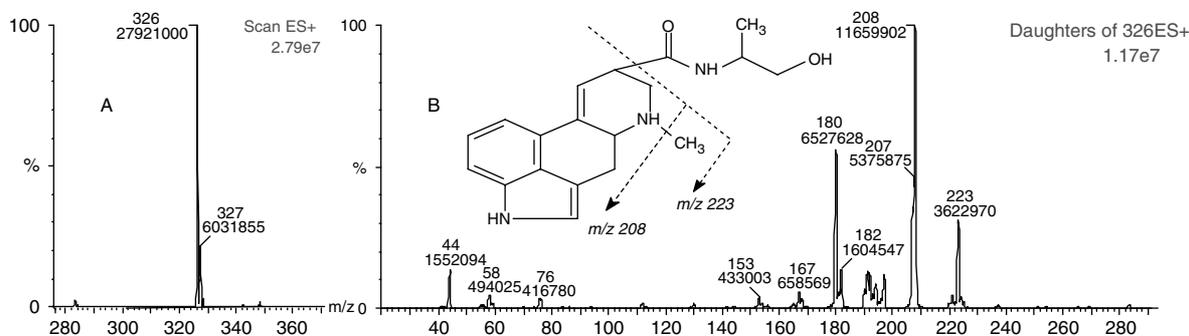


Figure 8. Ergonovine mass spectrometry. (A) ESI(+) mass spectrum of ergonovine infused as a 0.2 $\mu\text{g ml}^{-1}$ solution in acetonitrile–0.05% formic acid at 1.0 ml h^{-1} into the Quattro II mass spectrometer. (B) Fragment ion spectrum of the *m/z* 326 fragment seen in (A). Fragment ion principal peak assignments: *m/z* 223, loss of side-chain [H(C=O)NHCH(CH₃)CH₂OH]; 208, *m/z* 223 minus CH₃; 197, *m/z* 223 minus HC≡CH; 180, *m/z* 223 minus CH₂=NCH₃; 167, *m/z* 223 minus CH₂=CHNCH₃; 76, NH₃CH(CH₃)CH₂OH side-chain; 44, CH₂=NHCH₃. The inset shows the ergonovine structure protonated on the lysergic ring tertiary amine and indicating CID with the origins of the *m/z* 223 and 208 fragments.

absent from ergonovine and which supported the peptide ring system origin from the tertiary alcohol group. The other main fragment at m/z 180 on fragment ion analysis (Fig. 8(B)) was one of several fragments arising in turn from the initial cleavage at the carbonyl group to release a 223 u-sized fragment (initial cleavage to m/z 223 is shown in Fig. 8B (inset), along with m/z 208). Peak assignments for the principal peaks are given in the caption. Many of the peaks identified turned out to be a recurrent pattern with the ergot alkaloids family, as will be discussed later. $[M + H]$, $[M + 1 + H]$ and $[M + 2 + H]$ abundances were 100, 21.7 and 2.6%, respectively, which compared well with values of 100, 22.7 and 2.7% for a $C_{19}H_{24}N_3O_2$ compound.

Lysergol

Lysergol represents the reduced alcohol form of the lysergic acid-type clavine alkaloid. Figure 9(A) and (B) show the lysergol and fragment ion mass spectra, respectively, with interpretations of fragment ions in the caption. Cleavage to release the m/z 224 fragment and the demethylated m/z

240 are illustrated in Fig. 9B (inset) $[M + H]$, $[M + 1 + H]$ and $[M + 2 + H]$ abundances were 100, 20.5 and 2.3%, respectively, which compared well with values of 100, 18.9 and 1.8% for a $C_{16}H_{19}N_2O_1$ compound.

Lysergic acid

Lysergic acid represents the immediate synthetic precursor of the pharmacologically important natural ergot alkaloids, via amide linkage to the position-8 carboxy group. Figure 10(A) and (B) show the lysergic acid and fragment ion mass spectra, respectively, with interpretations of fragment ions in the caption. Note that intense dissociation of $CH_2=NHCH_3^+$ by cleavage of the D-ring affected the appearance of the rest of the spectrum, generally suppressing the relative ion abundance of other fragmentation events. As an example, cleavage of the D-ring to release the m/z 167 fragment is illustrated in Fig. 10B (inset). The m/z 44 ion was present throughout the surveyed compounds, but was more abundant in lysergol and lysergic acid under these conditions, and possibly indicated a stabilizing influence

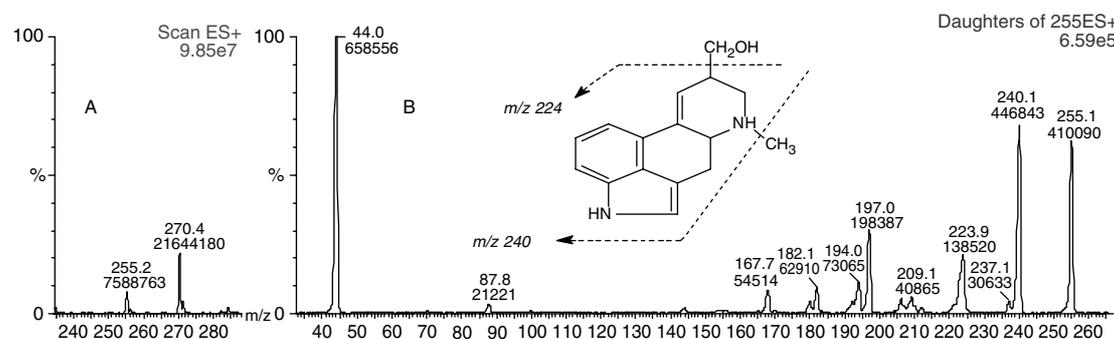


Figure 9. Lysergol mass spectrometry. (A) ESI(+) mass spectrum of lysergol standard infused as a $0.2 \mu\text{g ml}^{-1}$ solution in acetonitrile–0.05% formic acid at 1.0 ml h^{-1} into the Quattro II mass spectrometer. (B) Fragment ion spectrum of the m/z 255.2 fragment seen in (A). More information on the m/z 270 fragment is presented in a later section. Fragment ion principal peak assignments: m/z 255, $[M + H]^+$; 240, $[M + H]^+$ minus CH_3 ; 224, $[M + H]^+$ minus CH_2OH ; 209, m/z 240 minus CH_2OH ; 197, $[M + H]^+$ minus $\text{CH}_2=\text{CHCH}_2\text{OH}$; 182, m/z 240 minus $\text{CH}_2=\text{CHCH}_2\text{OH}$; 168, $[M + H]^+$ minus $\text{CH}_3\text{NCH}_2=\text{CH}(\text{CH})\text{CH}_2\text{OH}$; 88, $\text{CH}_3\text{NHCH}_2=\text{CHCH}_2\text{OH}$; 44, $\text{CH}_2=\text{NHCH}_3$. The inset shows the lysergol structure protonated on the lysergic ring tertiary amine and indicating CID with the origins of the m/z 224 and 240 fragments.

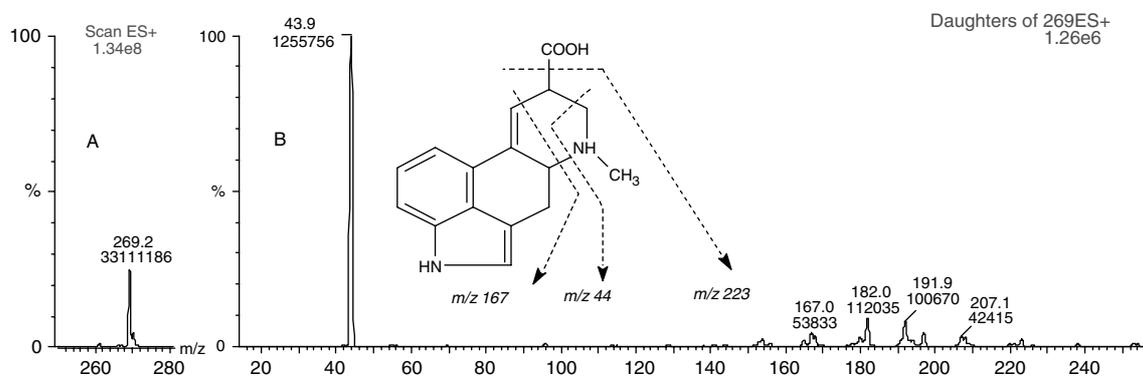


Figure 10. Lysergic acid mass spectrometry. (A) ESI(+) mass spectrum of lysergic acid standard infused as a $0.2 \mu\text{g ml}^{-1}$ solution in acetonitrile–0.05% formic acid at 1.0 ml h^{-1} into the Quattro II mass spectrometer. (B) Fragment ion spectrum of the m/z 269.1 fragment seen in (A). Fragment ion principal peak assignments: m/z 254, $[M + H]^+$ minus CH_3 ; 223, $[M + H]^+$ minus HCOOH ; 207, m/z 223 minus NH_2 ; 192, $[M + H]^+$ ring cleavage to release $\text{C}_4\text{H}_3\text{N}=\text{C}$; 182, m/z 254 minus $\text{CH}_2=\text{CHCOOH}$; 167, $[M + H]^+$ minus $\text{CH}_3\text{NHCH}_2=\text{CHCOOH}$; 44, $\text{CH}_2=\text{NHCH}_3$. The inset shows lysergic acid structure protonated on the lysergic ring tertiary amine and indicating CID with the origins of the m/z 223, 167 and 44 fragments.

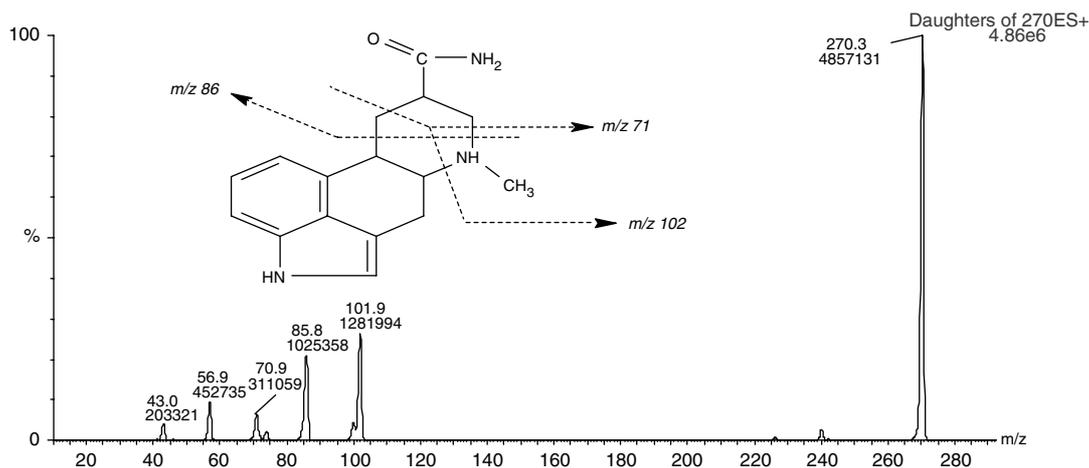


Figure 11. Recurrent mass spectral m/z 270 fragment ($C_{16}H_{20}N_3O$) seen on scans of some ergot alkaloid preparations, e.g. ergovaline, ergocryptine and lysergol, probably representing a common breakdown product or derivative. Seen at m/z 270, in this case during α -ergocryptine mass spectrometry with ESI(+)-MS of α -ergocryptine infused as a $0.2 \mu\text{g ml}^{-1}$ solution in acetonitrile–0.05% formic acid at 1.0 ml h^{-1} into the Quattro II mass spectrometer. The spectrum represents m/z 270 fragment ions. Fragment ion principal peak assignments: m/z 270, $[M + H]^+$; 240, $[M + H]$ minus CH_2NH_2 ; 102, $\text{CH}_3\text{NH}_2\text{CH}_2\text{CHC}(=\text{O})\text{NH}_2$ or variant; 86, $\text{C}_4\text{H}_8\text{NO}$; 74, $\text{C}_3\text{H}_8\text{NO}$; 71, $\text{C}_3\text{H}_5\text{NO}$; 57, possible $\text{C}_3\text{H}_5\text{O}$ fragment from m/z 102 for example; 43, $\text{CH}_3\text{N}=\text{CH}_2$. The inset shows one possible structure protonated on the lysergic ring tertiary amine and indicating CID with the origins of the m/z 102, 86 and 71 fragments.

of the amide function. $[M + H]$, $[M + 1 + H]$ and $[M + 2 + H]$ abundances were observed as 100, 18.2 and 2.9%, respectively, which compared well with values of 100, 18.9 and 2.0% calculated for a $C_{16}H_{17}N_2O_2$ compound.

Ergot alkaloid breakdown products

Certain ergot alkaloid preparations showed evidence consistent with the presence of contaminants arising presumably by hydrolysis or oxidation of the principal intact component, or perhaps by incomplete purification of a synthetic or natural product. The most significant such contaminant occurred consistently at m/z 270 (fragment ion spectrum shown in Fig. 11), which as an $[M + H]$ singly charged species indicated an uncharged compound molecular mass of 269. This odd value most likely indicated an odd number of N atoms, and since this species was seen with equivalent mass spectra in ergovaline, ergocryptine and lysergol, it most likely indicated a lysergic ring species with an additional nitrogen-containing functionality. Figure 11 (inset) offers a suggested structure, as a reduced form of lysergic acid amide. The caption gives the assignments of major peaks seen in the Fig. 11 fragment ion spectrum, in keeping with the general possibility of a lysergic acid ring system-derived structure. $[M + H]$, $[M + 1 + H]$ and $[M + 2 + H]$ abundances were observed for the lysergol-derived molecular mass 269 derivative as 100, 19.7 and 1.6%, respectively, which would seem to confirm the structure shown, or at least its molecular formula, which predicted values of 100, 19.3 and 1.8% for a $C_{16}H_{20}N_3O_1$ compound.

Discussion

This survey of principal ergot alkaloid compounds by ESI(+)-MS and- MS/MS allowed the assignment of fragments by comparisons of compounds with one another, and in turn revealed commonalities of fragmentation events.

Table 1 lists fragment ions found in common in the ergot alkaloid standards. This data set reveals that valine-derived ergopeptides (those with $R_1 = \text{isopropyl}$ in Fig. 1) display m/z 348, whereas alanine-derived ergopeptides (those with $R_1 = \text{methyl}$ in Fig. 1) display m/z 320. This assignment is supported by the absence of either peak in ergonovine, lysergol or lysergic acid, all three of which lack the peptide ring systems. A potential generic fragmentation mechanism is displayed in Fig. 12, shown here to account for major fragments of ergocornine ($[M + H]^+$, m/z 562) as an example. Cleavage to release the protonated lysergic ring system including the central amide linkage accounts for m/z 268. Otherwise, dehydration at the peptide tertiary alcohol occurs with intramolecular proton transfer from the lysergic D-ring tertiary amine or C-7 with generation of a tertiary carbonium ion at C-12' to account for m/z 544. Cleavage of the m/z 544 D-ring with loss of the R_2 group accounts for m/z 305 (common only to $R_1 = \text{isopropyl}$ compounds per Table 1). Alternatively, cleavage to release the entire lysergic ring system including the central amide linkage accounts for m/z 277. The m/z 544 dehydrate can undergo charge migration with concerted electron transfers to give a structure capable of cleavage to the major peak at m/z 348 or the minor fragment at m/z 195. This survey has accounted for ergocornine m/z 562, 544, 348, 305, 277, 268 and 195 ions by mechanisms directly applicable to the other compounds examined; m/z 223 and 208 are the other major ions generated as shown schematically in Figs 2, 3 and 5–8, with m/z 208 apparently arising by violation of the even-electron rule as demonstrated in Fig. 4.

The positing of an intramolecular proton shift for generation of a carbonium ion during dehydration was suggested above to account for the difficulty that simple direct scission of the five-membered lactam ring E yields a fragment 2 u

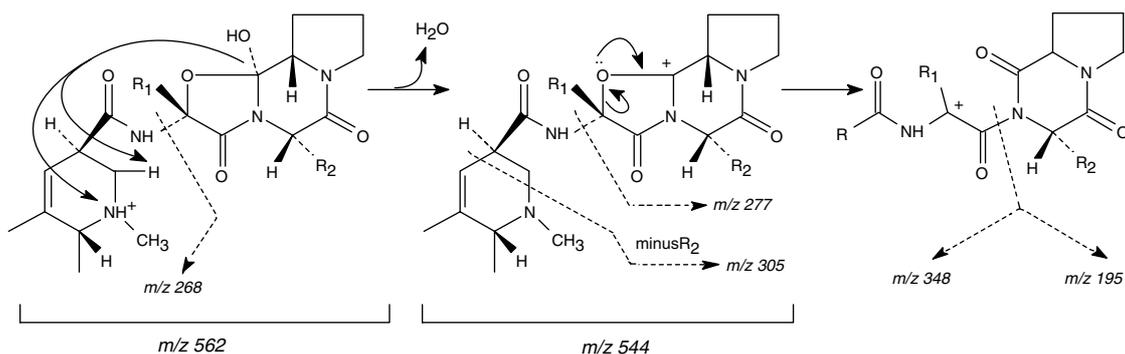


Figure 12. Possible scheme for principal fragmentation mechanisms, in this case to account for principal peaks in ergocornine protonated on the lysergic ring tertiary amine as an example ($R_1 = \text{CH}_2(\text{CH}_3)_2$, $R_2 = \text{CH}_2(\text{CH}_3)_2$). Protonation at the lysergic D-ring tertiary amine provides $[\text{M} + \text{H}]^+ = m/z 562$. Cleavage at the central amide releases the lysergic ring system as $m/z 268$; alternatively, dehydration at the tertiary alcohol via intramolecular proton extraction induces charge migration with generation of a tertiary carbonium ion, $m/z 544$; the dehydrate cleaves to release $m/z 277$ or 305 (with release of R_2), or rearranges with migration of charge to provide an intermediate that cleaves to account for $m/z 348$ or the minor fragment at $m/z 195$.

Table 1. Fragments ions common to ergot alkaloid standards: \times = fragment found as a peak in compound's fragment ion mass spectrum (R_1 and R_2 substituents refer to Fig. 1)^a

<i>m/z</i>	1	2	3	4	5	6	7	8	Assignment
R_1	me	me	ipr	ipr	ipr	(me)	-	-	
R_2	ipr	bzl	ipr	ibu	bzl	-	-	-	
$[\text{M} + \text{H}] - 18$	\times	\times	\times	\times	\times				Loss of water [peptide ring system tertiary alcohol]
$[[\text{M} + \text{H}] - 18] - 28$	\times	\times	\times	\times	\times				Loss of CO from dehydrate
$[[\text{M} + \text{H}] - 18] - 267$	\times	\times	\times	\times	\times				Loss of [lysergic ring-CONH ₂] from dehydrate
$[[\text{M} + \text{H}] - 18] - 224$	\times								Loss of [lysergic ring] from dehydrate
348			\times	\times	\times				Cleavage within and loss of most of peptide ring system (in amide and at ether) to include isopropyl group
320	\times	\times							Cleavage within and loss of most of peptide ring system (in amide and at ether) to include methyl group
305			\times	\times	\times				Loss of R_2 from dehydrate plus cleavage of lysergic D ring to retain first 2 carbons
291				\times					Loss of R_1 and lysergic ring system from dehydrate
277	\times		\times						Loss of R_1 and lysergic ring system from dehydrate
268	\times	\times	\times	\times	\times				[Lysergic ring system-C=O-NH ₂] + H
251	\times	\times	\times	\times	\times				$m/z 268$ minus NH ₃
225	\times	\times	\times	\times	\times				Peptide ring system released by cleavage of M+H central amide group, minus alkyl or aralkyl side groups, or +H ₂ variant of $m/z 223$
223	\times	\times	\times	\times	\times	\times		\times	$[\text{M} + \text{H}]$ minus [peptide side chain-NH ₂ -HC=O]
208	\times	\times	\times	\times	\times	\times		\times	$m/z 223$ minus CH ₃
197	\times	$m/z 223$ minus CH≡CH							
180	\times	$m/z 223$ minus CH ₂ =NCH ₃							
167			\times			\times		\times	$m/z 223$ minus CH ₂ =CHNCH ₃
70	\times	\times	\times	\times	\times				Peptide ring system pyrrole [C ₄ H ₈ N]
44	\times	CH ₂ =NHCH ₃							

^a Compounds: 1 = ergovaline; 2 = ergotamine; 3 = ergocornine; 4 = ergocryptine; 5 = ergocryptine; 6 = ergonovine; 7 = lysergol; 8 = lysergic acid. Abbreviations: me = methyl; ipr = isopropyl; ibu = isobutyl; bzl = benzyl.

greater than required in the case of each ergotoxin. Molecular modeling with Hyperchem software was performed to provide a geometry-optimized three-dimensional structure for ergocornine as an example, and this revealed that the C-7 α -proton and the hydroxyl group oxygen atom are relatively sequestered and only 1.831 Å apart, making such

a transfer highly plausible (structures are shown in Fig. 13). This contrasts with crystallographic findings from studies of dehydrated crystals, which suggest rigid central amide O to C-12' OH hydrogen bonding as a general ergopeptide motif;²⁰ however, the new proposed arrangement requires simple rotation of the C-2' carbon-nitrogen bond to depart

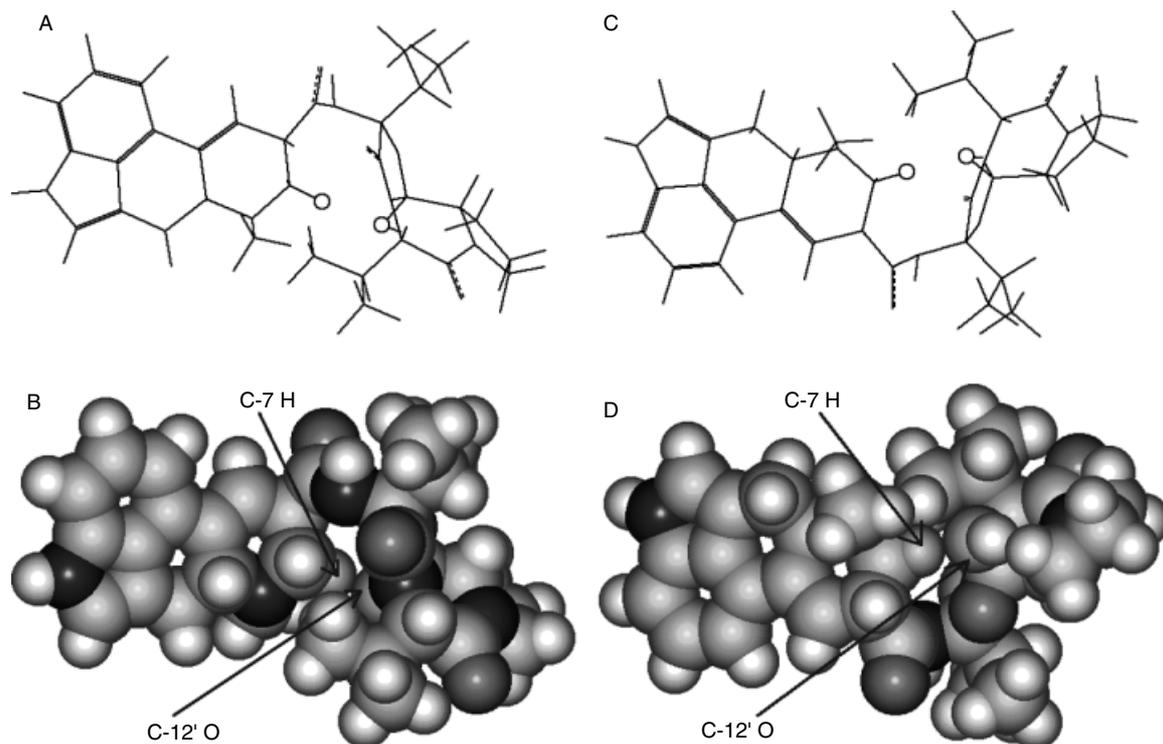


Figure 13. Demonstration of proximity of lysergic D- and peptide F-rings. Geometry-optimized structure of ergocornine provided by Hyperchem software. (A) and (B) show stick figure and space-filling views from the same side of the molecule, with the lysergic ring system to the left and oriented similarly to the graphical representation in Fig. 1. (C) and (D) show the same from the opposite side (rotation along a latitudinal axis). Circles in (A) and (C), and arrows in (B) and (D) indicate the C-7 α -proton (C-7 H) and the C-12' hydroxyl oxygen atom (C-12' O), which are separated by a calculated distance of only 1.831 Å.

from the hydrogen bonding arrangement suggested by the x-ray structure. Further rotation around the bond from C-8 to the amide carbonyl group can also bring the C-12' hydroxyl oxygen atom and D-ring amine nitrogen atom within 2–3 Å, implying that ammonium proton transfer to C-12' OH is likely to be at least an equivalent possibility.

The m/z 223 fragment observed and illustrated in Figs 2, 3 and 5–8 requires cleavage at the C-8 carbon atom, and a potential fragmentation pathway is suggested in Fig. 14. Molecular modeling indicated proximity of the carbonyl oxygen and quaternary amine proton only following epimerization at C-8 (data not shown).

With regard to other common fragments in Table 1, strong m/z 277 fragments from ergovaline and ergocornine, and also m/z 291 from ergocryptine, seemed to correlate well with loss of R₁ and the lysergic ring system from corresponding dehydrates; this transition was not found for the R₂ = benzyl compounds. There is apparently an alternative structure for m/z 277 that includes the lysergic ring system, since minor m/z 277 peaks were seen from ergotamine and ergocryptine and m/z 277 was seen as a parent to m/z 208 in ergocornine (Fig. 4) and ergocryptine. The m/z 225 fragment was originally considered as deriving from the peptide ring system owing to its presence only in the spectra of peptide ring system-containing compounds, and conversely its absence in ergonovine, lysergol and lysergic acid. However, its occurrence as a parent ion for m/z 208 (Fig. 4) implies inclusion of the lysergic ring system, with the simplest interpretation being a +H₂ variant of m/z 223

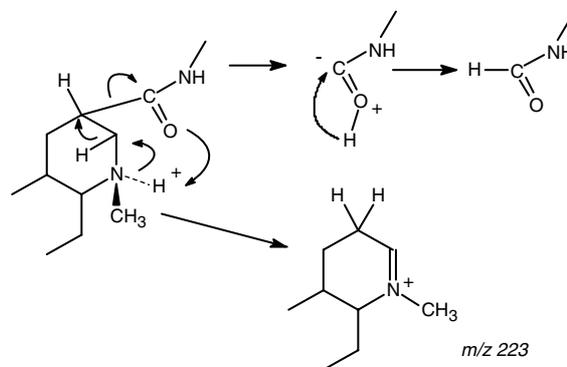


Figure 14. Possible fragmentation pathway to the m/z 223 fragment seen in Figs 2, 3 and 5–8. Protonation of the lysergic ring system tertiary amine imparts a positive charge; intramolecular proton transfer to the central amide linkage carbonyl group results in a positively charged ammonium group upon electron transfer. Subsequent or simultaneous cleavage to release the amide and adjacent protein group results in a leaving group with temporary charge separation that resolves by shift of a proton to give a neutral structure.

whose origin depends mechanistically upon the peptide ring system, particularly since ergonovine lacks both a peptide ring system and m/z 225. The m/z 197 fragment can be accounted for by cleavage and release of a CH≡CH neutral fragment from m/z 223 simply from the D-ring. The m/z 44 peak is of relatively minor intensity in most of the standards,

Table 2. Predicted nominal [M + H] and fragment ions for ergot alkaloids

Compound	M_r	[M+H] (m/z)	Fragments (m/z)
Dihydroergocristine	611	612	225, 210
Ergocristine	609	610	223, 208
Ergostine	595	596	223, 208
Ergotamine	581	582	223, 208
Dihydroergocryptine	577	578	225, 210
Ergocryptine	575	576	223, 208
Dihydroergocornine	563	564	225, 210
Ergotamine dehydrate	563	564	223, 208
Ergocornine	561	562	223, 208
Ergoptine	561	562	223, 208
Ergonine	547	548	223, 208
Ergosine	547	548	223, 208
Ergovaline	533	534	223, 208
Nicergoline	483	484	214, 199
Cabergoline	451	452	251, 210
Ergonovine	325	326	223, 208

and has been assigned a $\text{CH}_2=\text{NHCH}_3^+$ fragment arising from the D-ring. This fragment therefore unites the peptide ring-bearing ergotoxins with ergonovine and the clavine-type ergots, especially lysergol and lysergic acid where the m/z 44 fragment is of significant abundance (Fig. 10). The lack of m/z 44 from the molecular mass 269 species probably results from its specific structure, most likely the electronic configuration of the D-ring. The m/z 70 fragment is found only in those compounds with the peptide ring system, supporting its assignment as arising from two cleavages at an amide and a C—C bond to release a pyrrole ring. One curious finding concerned the differences in scission events between lysergol and lysergic acid, presumably guided by the differing oxidation states of the side-chain: the series of fragments at m/z 223, 208, 180 and 167, seen in lysergic acid and most likely having the same resultant structures as those in the other alkaloids, become m/z 224, 209, 180 and 168 in lysergol, accounting for the absence of most of these peaks in its list of common fragments (Table 1).

The understanding of ergot alkaloid fragmentation as summarized in Table 1 enables predictions to be applied to compounds with closely related structures. Table 2 summarizes primary cleavages expected for a series of peptide- and non-peptide-type ergot alkaloids seen in nature and related to and including those examined here. Although other fragments could also be included, this list focuses on those arising from loss of the peptide side-chain and its associated $\text{NH}_2-\text{HC}=\text{O}$ linkage as described in Figs 2, 3 and 5–9, along with the corresponding loss of methyl (–15) from the resulting fragment. This list could provide the basis for multiple reaction monitoring (MRM) detectability in any tandem quadrupole instrument equipped with an electrospray inlet, whether the aim is quantitation of known compounds such as the standards examined here or the detection of new compounds including those in Table 2. Application of these approaches to MRM detection of naturally occurring ergot alkaloids will be reported in a forthcoming paper.

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