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RESEARCH ARTICLE

ESI-Mass spectrometric and HPLC elucidation of a new ergot alkaloid from perennial ryegrass hay silage associated with bovine reproductive problems

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Abstract

This case report involves four dairies in the Willamette Valley, Oregon, which experienced reproductive problems associated with the presence of a large, previously unidentified, peak eluting at 5 min in a standard ergovaline high-performance liquid chromatography assay of perennial ryegrass silage fed to those animals. Mycotoxin analysis of the silage was negative, as was serological screening of the herds for infectious bovine rhinotracheitis, bovine diarrhea virus and *Leptospirosis*, including culturing of urine for *Leptospira hardjo hardjobovis*. Prolactin concentrations were low in most cattle, consistent with ingestion of ergot alkaloids. We believe that this peak represents a novel ergot alkaloid-related compound due to its extractability with Ergosil, its detectability due to fluorescence, and its chromatographic retention between ergovaline (mw = 533) and ergotamine (mw = 581). Its molecular weight was calculated as 570 owing to the predominance of a *m/z* 593.5 ion in the full scan ESI(+)MS and its deduced tendency to complex with Na^+ (as *m/z* 593) or K^+ (as *m/z* 609) ions. We offer rationales for elucidation of the structure of this compound, with the closest starting point comprising an m.w. of 566—a fructofuranosyl-(2-1)-O-beta-D-fructofuranoside derivative of 6,7-secoergoline from *Claviceps fusiformis*. This m.w. requires modifications, such as reduction of two double bonds in the secoergoline component to give the target 570 m.w. Despite the lack of a definitive structure, the analysis herein provides a starting point for eventual elucidation of this apparently new ergot alkaloid, and to guide and encourage further investigation as to its association with endophyte toxicosis in livestock.

Keywords: Ergot alkaloids, silage, ryegrass, dairy cows, reproductive problems

Introduction

Perennial ryegrass (*Lolium perenne*) is a cool-season grass which is infected with the endophytic fungus *Neotyphodium lolii*. This symbiotic endophyte infection confers benefits to the plant such as insect resistance, growth enhancement and drought tolerance (Joost, 1995), thereby decreasing the need for pesticides, fertilizers and irrigation. The fungus provides some of these benefits through production of insect-repelling alkaloids, particularly peramine; other compounds are also synthesized, including lolitrem and ergot alkaloids (Cheeke, 1998). Unfortunately, cattle and other herbivores that ingest these alkaloids experience deleterious effects, which have been grouped under the umbrella term "endophyte toxicosis," when endophyte-infected grasses are grazed or fed as hay/silage (Oliver, 2005). The alkaloid lolitrem B is responsible for the neurological syndrome known as "ryegrass staggers" which involves a reversible tremoring response in the skeletal musculature of affected animals due to inhibition of large

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washed with a chloroform:acetone mixture followed by methanol. The ergot alkaloids were eluted with methanol and concentrated by nitrogen evaporation before analysis by HPLC. A polymeric divinyl benzene column (Jordi RP SM-500A, 5μ (150 mm × 4.6 mm)) was employed with a mobile phase of acetonitrile/2.5 mM ammonium carbonate (70/30 v/v) and run at a flow of 1.0 mL/min. Detection was by fluorescence with excitation and emission wavelengths of 250 and 420 nm, respectively.

A new peak, eluting at approximately 5 min in the ergovaline HPLC assay, appeared in perennial ryegrass silage samples at varying concentrations (Figure 1). To concentrate and purify this peak, samples with a large concentration of this compound were extracted using the ergovaline methodology, pooled, dried under a nitrogen stream at 50°C and reconstituted in 0.5 mL methanol. The pooled sample was then vortexed, sonicated and centrifuged for 5 min at 2000 rpm. The supernatant was injected onto the HPLC system and the peak eluting at 5 min was captured by manual fraction collection. The corresponding fraction was dried under a nitrogen stream at 50°C, reconstituted with 1 mL methanol, vortexed, sonicated and centrifuged for 5 min at 2000 rpm. The final supernatant was stored in an amber vial in the freezer (−20°C) until mass spectral analysis was performed.

ESI(+)-ion trap mass spectrometry

Spectra were first obtained on a LCQ Classic ion trap mass spectrometer (Thermo Finnigan, Waltham, MA) equipped with a custom-designed electrospray inlet consisting of a 30 micron i.d. steel capillary heated at 170°C and operated at 2.7 kV. Solvent flow was controlled by a HPLC system consisting of a Waters (Milford, MA) Automated Gradient Controller with two Waters 515 HPLC pumps and a Rheodyne 8125 injector. The HPLC solvents used were water and acetonitrile, each containing 0.1% acetic acid and 0.01% trifluoroacetic acid eluting under isocratic conditions in a 50:50 ratio. Samples were introduced to the spectrometer by loop injection with data acquisition in the positive ion mode. MS/MS analysis was performed on MS peaks with the highest relative intensity.

ESI(+)MS/MS

A Micromass Quattro II ESI(+)-MS/MS (Beverly, MA) was calibrated with polyethylene glycol 400 standard and tuned for positive ion mass spectrometry by direct

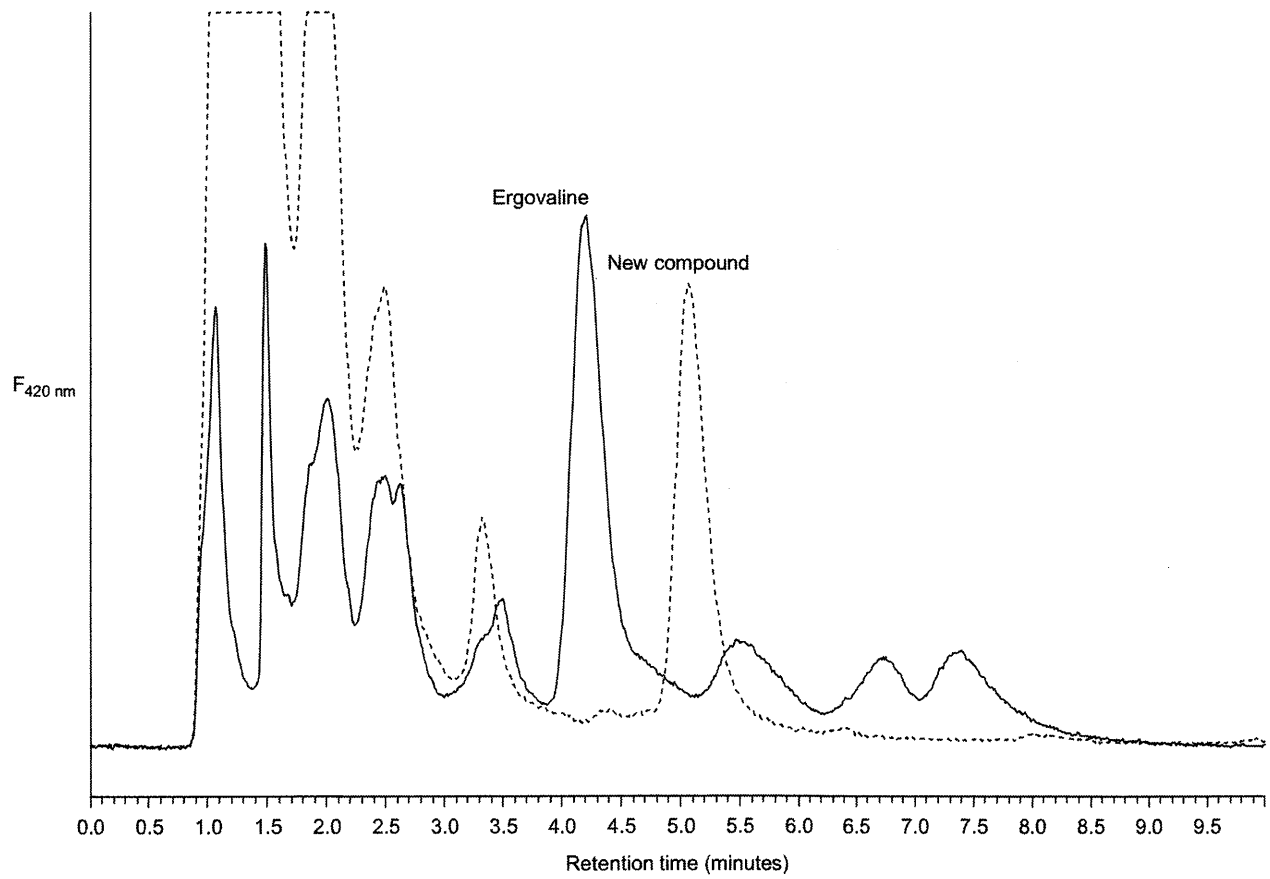

Figure 1. HPLC chromatogram of an extract of fescue straw control (dark solid line) overlaid with that of a silage sample containing the new peak at 5 min (gray dotted line). Ergovaline elutes at 4.1-min retention time, and ergotamine standard (data not shown) at 6.1-min retention time; the unknown peak under investigation elutes at 5.1-min retention time. The y-axis shows relative fluorescence emission at 420 nm following excitation at 250 nm.

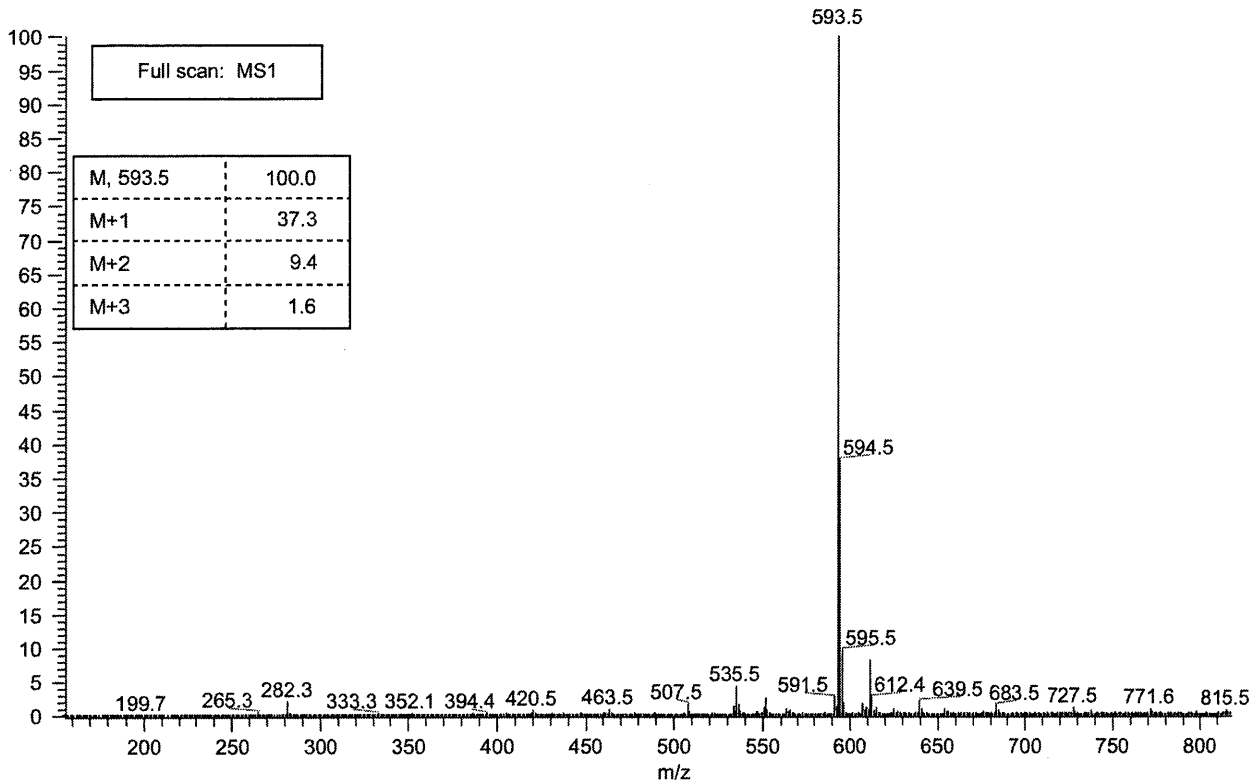

Figure 2. Full scan ESI(+) ion trap MS of isolated putative ergot-related compound at ~ 5-min retention time. Scans were acquired from *m/z* 150–2000 and full scale abundance (y-axis) was 1.19×10^7 intensity. The inset shows the relative abundances of M+1, +2 and +3 isotopes relative to the principal *m/z* 593.5 compound. (See colour version of this figure online at www.informahealthcare.com/txm)

(Bosken et al., 2000) and macrocyclic lactones (Lehner et al., 2009). Verification of such complexes can involve titration with H^+, Li^+ or NH_4^+, or with other alkali metals (Bosken et al., 2000), self-consistent interpretations of related compounds (Lehner et al., 2009) and/or product ion scans (see below), or direct observation of Na^+ or K^+ ions, *m/z* 23 and 39, respectively (Lehner et al., 2009). We chose the latter two approaches for the work presented here.

Examination of the *m/z* 183 component seen in Figure 4 provided the product ion spectrum in Figure 5. The *m/z* 39 peak seen in this spectrum suggests potassium adduct formation. Making the simple subtraction *m/z* 183 – 39 = 144 and scanning the searchable version of the Merck Index for this molecular weight principally revealed the low molecular weight organic acid caprylic acid (octanoic acid) as a strong possibility, along with its isomers including ethyl caproate and valproic acid as alternative candidates. Principal peaks *m/z* 115, 101 and 65 agree ± 1 amu with the Wiley published electron impact mass spectrum of potassium-free octanoic acid. The presence of caprylic acid would not be unexpected, owing to its use as a fermentation stimulant and silage preservative (Abel et al., 2002).

Knowledge of the presence of alkali metal adducts aided in interpretation of the principal components of interest at *m/z* 539 and 609. Product ion scans of these ions as shown in Figure 6 revealed the presence of sodium (*m/z* 23) and potassium (*m/z* 39), respectively. Since both 539 – 23 and 609 – 39 give the same value of 570, this strongly suggested that these components are related and involve an uncharged molecular weight of 570. Table 1 lists components seen by ESI(+)MS/MS and examined by daughter ion analysis. *M/z* 183, 593 and 609 were the principal components seen to contain alkali metal components, and the resultant inferred molecular weights are tabulated.

Mass spectral analysis of the new compound as a sodium adduct

Table 2 lists mass spectral fragments seen as a result of ESI(+)-ion trap MS in comparison to those seen by ESI(+)MS/MS, both for the *m/z* 593 species as well as for the likely related *m/z* 609 component. Values calculated as arising by subtraction of the sodium (mass 23) or potassium (mass 39) components are shown in the shaded areas, and these calculations provide excellent evidence of unity between these disparate measurements, as seen specifically in the *m/z* 593 derived fragments (*m/z* 593, 565, 533, 476, 461, 433) from the two different instruments; in the observed fragments *m/z* 565, 489, 95, 81 and 60 seen on comparison of *m/z* 593 and 609 derived fragments from ESI (+)-MS/MS; and as seen by alkali metal subtracted fragments (*m/z* 526, 492, 482, 450, 438, 424, 382, 235, 197) on comparison of all three.

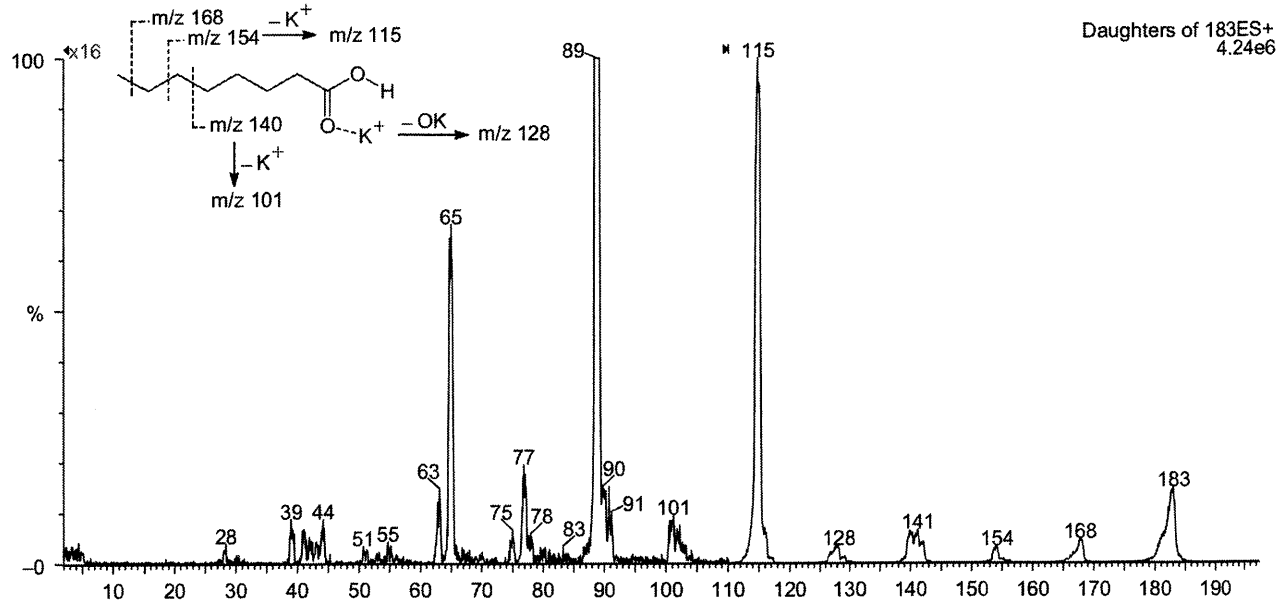

Figure 5. Product ion scan of *m/z* 183 seen in Figure 4. Note 16× enhancement of *m/z* 0–110 range. The inset shows an interpretation of the spectrum as arising from a caprylic acid-K^+ adduct, made likely by the presence of the *m/z* 39 potassium peak. (See colour version of this figure online at www.informahealthcare.com/txm)

Mass spectral elucidation of the new peak structure

High resolution mass spectrometric instrumentation was unavailable for this work, so the following interpretations should be considered preliminary. Note, however, that our labs have had reasonable success in interpreting mass spectral fragments from low resolution, e.g. quadrupole, instruments, including data on isotopic abundances (Lehner et al., 2004a, 2004b, 2009).

General ergot alkaloid structures are summarized in Figure 7. Our earlier work discerned certain recurring mass spectral fragmentation motifs in the ergot alkaloids (Lehner et al., 2004a, 2005), enabling us to predict structures of new compounds. Specifically, we had considerable success in assigning structures to numerous unique or newly discovered ergopeptine alkaloids by unique recombination of amino acid R-groups already known to be incorporated into ergopeptines (Lehner et al., 2005). We decided to reapply this method as an initial approach and consider all possible genetic code-encoded amino acids plus ethyl as possible origins of R-groups in ergopeptines (R_1 and R_2 in structure C, Figure 7). Values of interest based on this type of calculation are listed in Table 3. None of the combinations directly provided m.w. 570, but even mws 574 (intact alkaloid) or 572 (dehydrate) could be considered as starting points for structure elucidation. Figure 8 shows an example of a putative ergot structure derived from application of reasoning with amino acid R-groups, in this case a lys/val structure making it an analog of the known ergot alkaloid ergovaline. However, although this approach may be capable of deriving unique candidates capable of fulfilling the expected 570 m.w. target, it suffers from two major drawbacks: (i) lack of precedent for such unusual R-groups in ergopeptines, and (ii) lack of "diagnostic" mass spectral fragments reflective of ergopeptine-related compounds, as summarized by Lehner et al. (2004a) and including values such as *m/z* 208 and 223, or 210 and 225 for those involving saturation of the 9,10-bond. This lack of diagnostic fragments was supported by parent ion scans for these ions in the silage derived material which revealed no candidate high m.w. components (data not shown).

We thus took several new approaches to deciphering the structure of the unique 570 m.w. compound, including (i) comparison of molecular weight and mass spectrometric fragments to a comprehensive mycotoxin database; (ii) comparison to a comprehensive ergot alkaloid database; (iii) mass spectrometric fragment analysis; and (iv) consideration of other chemical and spectral properties.

The clinical situation strongly suggested that the high molecular weight component of m.w. 570 is a unique ergot alkaloid, particularly since no other candidate ergot alkaloids were identifiable. The uniqueness of this compound was verified by reference to a comprehensive mycotoxin/fungal metabolite database (Nielsen & Smedsgaard, 2003); no entries in that 474-compound database provided relevant compounds in the 569–571 m.w. range, nor did any mass spectral data entries disclose ESI(+)MS fragments *m/z* 593, 533, 461 or 235 (i.e. principal fragments in Figure 3) in any manner that seemed to reflect on the discovery reported here.

We next scanned the ergot-specific database of Flieger and coworkers (Flieger et al., 1997). The best known ergot alkaloid skeletal structures including 6,7-secoergoline, ergoline, ergopeptine and ergopeptam basic skeletons are

Table 1. ESI(+)MS/MS analysis: revelation of Na^+ or K^+ adducts by examination of daughter ion spectra and inspection for *m/z* 23 or 39 fragments, respectively.

Molecular ion	Na/K evident	Likely m.w.
183	K	144
316	None	315
342	None	341
460	None	459
488	None	487
535	None	534
593	Na	570
609	K	570
889	Not examined	888 or 866

summarized in Figure 7. All possible known ergot alkaloid structures that could be condensed from this review are summarized in Table 4. The shaded section of Table 4 indicates the region of high molecular weight ergot alkaloid structures, including hypothetical demethylated or dehydrated variants. The closest possibilities wound up being fructofuranosyl-(2-1)-O-beta-D-fructofuranoside derivatives of 6,7-secoergoline derived from *Claviceps fusiformis* and their demethylated analogs, labeled W, X, Y and Z in Table 4. An example of such a structure, after Flieger et al. (1990) is shown in Figure 9. As a first approximation, for example, the hypothetical demethylated 566 m.w. variant X of the 580 m.w. starting compound Z would require reduction of two double bonds to give 570 m.w., shown as the first candidate structure A in Figure 10.

We believe that the difructosyl structures at 578 and 580 m.w. (Y and Z in Table 4) provide a valuable clue in that they are the only even m.w. compounds in the shaded high m.w. range of Table 4. With the exception of quaternary amines, the nitrogen rule of organic chemistry requires that compounds comprised of C,N,O,S,F,Cl, and Br have even molecular weights if the N-number is even (including zero) and odd m.w. if the N-number is odd (McLafferty & Turecek, 1993). Analogs of structures Y or Z therefore have the advantage of being of sufficiently high molecular weight without introducing an odd number of N-atoms as in the ergopeptines and ergopeptams.

The compounds of Table 4 comprise the atoms C, H, N, S and O, with a single entry at 593 m.w. offering an S atom. Consideration of the likelihood that the unique 570 m.w. species comprises only C, H, N and O atoms is supported by the lack of significant M+2 values in the relative isotopic contributions for *m/z* 593, ruling out possible S, P or halide functionalities, although S becomes a special consideration as discussed below. The relatively high M+2 for *m/z* 609 is not unexpected, owing to the considerable M+2 contribution of 7% for potassium, in contrast to lack of isotopic contributions from sodium. In any case, calculation of all possible molecular formulae with constraints as imposed according to the materials and methods for the Formula Calculator yielded 493 entries with m.w. 570 ± 0.5 and provided a range of M+1 isotopic contributions from 32 to 42%.

Structure A in Figure 10 meets the self-imposed stipulations of tight M+1 and M+2 isotopic contributions and therefore comparability to the mass spectrometric data. It is therefore a possible structure for the putative m.w. 570 ergot alkaloid similar to frucofuranosylated chanoclavine compounds seen in *C. fusiformis* (Flieger et al., 1997).

Mass spectrometric fragment analysis was the next step in dissecting the structure of the unique ergot alkaloid. Loss of 60 amu as in *m/z* 593→533 (Figure 3 and Figure 6, top) is suggestive of acetylation, with loss of neutral acetic acid as an explanation for the 60 loss, and a review of acetylated electron impact mass spectra reveals the loss of 60 as a predictable feature, e.g. 4,4-dimethyl-cholest-7-en-3-ol, 3-beta-acetate (NIST98 Library of EI-mass spectra). There is precedent for acetylation in fumigaclavine A from *Aspergillus fumigatus* and its isomer roquefortine A from *Penicillium roquefortii* (Flieger et al., 1997), but these are only m.w. 298 in Table 4. Fumigaclavine C adds an additional isoprenoid R-group (+68) but still falls far short at m.w. 366.

Supportive of a fructofuranosyl-(2-1)-O-beta-D-fructofuranoside ergot alkaloid is the alternative possibility that loss of 60 amu may occur as CH(OH)-CH(OH) fragments from fructosyl structures. Review of NIST98 EI-mass spectra shows the *m/z* 60 fragment in 1-O-methyl-D-fructose, for example. In addition, the 1,3,4,5,6-pentakis-O-(trimethylsilyl) derivative of D-fructose contains the significant *m/z* 204 consisting of the bis(trimethylsilyl) derivative of the CH(OH)-CH(OH) fragment further indicating the likelihood of such a fragmentation.

The spectral property of fluorescence enabled initial detection of the new unique ergot alkaloid and must be taken into consideration. Although there is no specific program by which fluorescence can be precisely predicted, structural motifs associated with fluorescence have nonetheless long been known. Several generalizations as to the structural requirements for fluorescence in solution include (i) an aromatic nucleus substituted by at least 1 electron-donating group, and (ii) a conjugated unsaturated system capable of a high degree of resonance (Duggan et al., 1957). The lysergic, i.e. unsaturated ergoline, ring system of ergots is generally accepted as the source of fluorescence in ergopeptines, and saturation of the 9,10 double bond eliminates fluorescence (Gyenes & Szasz, 1955; Wichlinski & Trzebinski, 1963; Mago-Karacsony et al., 1979). Although the Figure 10A candidate structure fulfills molecular weight and isotopic contribution requirements as well as deriving from literature precedents, it unfortunately carries the signal disadvantage of eliminating any double bond conjugation with the aromatic ring, thereby simultaneously eliminating chances for fluorescence. The alternative structure in Figure 10B allows reintroduction of the indole double bond, simply by eliminating a methyl group and substituting with a hydroxyl to maintain the target 570 m.w.; however, this is unlikely sufficient

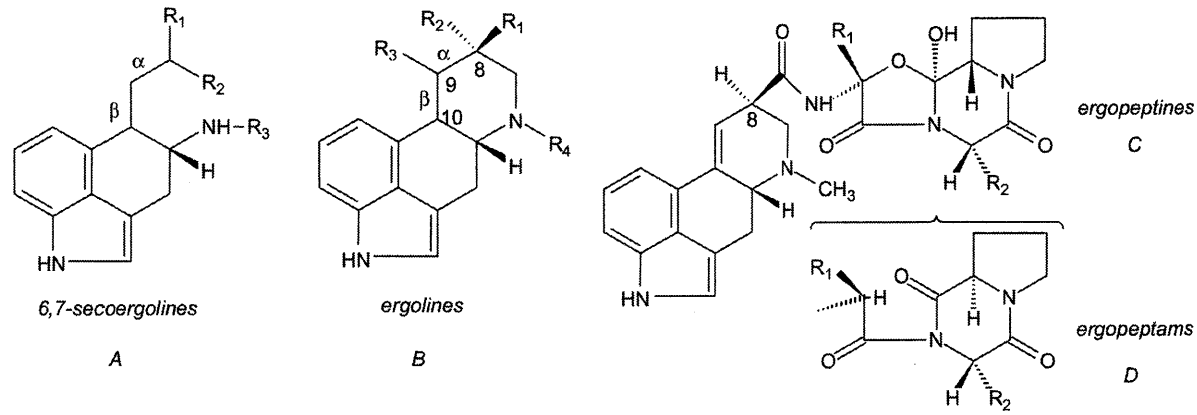

Figure 7. General ergot alkaloid structures, condensed from Flieger et al. (1997), including 6,7-secoergolines (A), ergolines (B), and lysergine derivatives ergopeptines (C) and ergopeptams (D). R-group components are discussed in the text and in Table 4.

Table 3. Hypothetical ergopeptine substituents from consideration of 20 amino acid R-groups plus ethyl as possibilities; shaded areas indicate values surrounding m.w. 570 both for calculated m.w.'s as well as for dehydrates.

2′ [or 5′] substituent	5′ [or 2′] substituent	Calculated m.w.	Dehydrate m.w.
phe	gly	567	549
cys	thr	567	549
cys	cys	569	551
His	ala	571	553
pro	leu/ileu	574	556
His	ethyl	585	567
His	ser	587	569
Leu/ileu	leu/ileu	589	571
Gln	pro	589	571
Lys	pro	589	571
Arg	ala	590	572
Gln	val	590	572
Lys	val	590	572
Asn	leu/ileu	590	572
Glu	pro	590	572
Glu	val	591	573
asp	leu/ileu	591	573
asn	asn	591	573

the target 570 m.w. Candidate C required an additional hydroxyl group to meet the target 570 m.w., whereas candidate D replaces the added hydroxyls with a single thiol group. Ironically, in order to accommodate a fluorescent structure (Figures 10C or D), the sought after agreement on isotopic abundances must be circumvented.

Therefore, in summary, the Figure 10D structure may at this time be the most satisfying possibility as it (i) takes advantage of the high m.w. fructofuranosyl group without introducing an odd number of N-atoms; (ii) does not require invocation of unusual amino acid substitution into an ergopeptine structure; (iii) derives the basis of its high molecular weight structure from model compounds in the literature; and (iv) most importantly, it introduces the double bonds at positions 2,3 and 9,10 most crucial for fluorescence in the molecule.

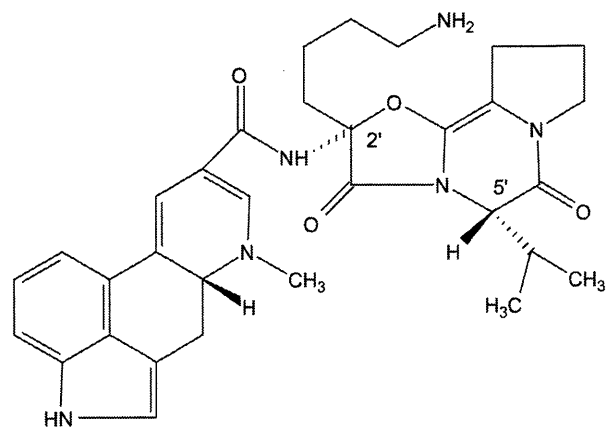

Figure 8. An example of an unusual ergopeptine structure of 570 m.w. related to ergovaline by substitution of its 5′-methyl group with a lysine R-group, by dehydration of the peptide ring system, and by introduction of an additional double bond into the lysergic ring system at position 8.

Discussion

The unique 5.0-min RT compound is suggested to be an ergot alkaloid-related compound by virtue of its association with clinical ergot-related symptomologies, its extractability with Ergosil, its detectability with a fluorescence detector ($\lambda_{ex}=250$; $\lambda_{em}=420$), and its chromatographic retention in the region between ergovaline (mw=533) and ergotamine (mw=581). Its apparent molecular weight is 570 owing to the predominance of the *m/z* 593.5 ion in the full scan ESI(+)MS (Figure 2) and its deduced tendency to complex with Na^+ or K^+ ions. We have done our best to offer structural rationales for this compound (Figures 8 and 10) and next discuss the evidence, supportive or otherwise, for each structure.

The stimulus for substituting alternative amino acid R-groups into the basic ergopeptine ring system (Table 3) derived from earlier successes in interpreting new or novel ergotoxins (Lehner et al., 2004a, 2005). Those were principally performed with recombinations of the known 2′-substituents of alanine, alpha-aminobutyrate

Table 4. Continued.

R_1	R_2	R_3	R_4	DB	Str	Isomers	MW	dehydrate	demethyl
CH_2CH_3	$CH(CH_3)_2$				C		547	529	533
$CH(CH_3)_2$	CH_2CH_3				C		547	529	533
$CH(CH_3)_2$	$CH_2CH(CH_3)_2$				D		559	541	545
$CH(CH_3)_2$	$CH(CH_3)CH_2CH_3$				D		559	541	545
CH_2CH_3	$CH_2CH(CH_3)_2$				C		561	543	547
CH_2CH_3	$CH(CH_3)CH_2CH_3$				C		561	543	547
$CH(CH_3)_2$	$CH(CH_3)_2$				C		561	543	547
CH_3	$CH_2C_6H_5$				D		565	547	551
$CH(CH_3)_2$	$CH_2CH(CH_3)_2$				C		575	557	561
$CH(CH_3)_2$	$CH(CH_3)CH_2CH_3$				C		575	557	561
$CH(CH_3)_2$	$CH(CH_3)CH_2CH_3$				C		575	557	561
$CH(CH_3)_2$	$CH_2CH(CH_3)_2$				C		575	557	561
$CH_2O(C_6H_{10}O_4)O(C_6H_{10}O_4)OH$	n.a.	H	CH_3	α	B		578 [Y]	560	564 [W]
CH_2CH_3	$CH_2C_6H_5$				D		579	561	565
$CH_2O(C_6H_{10}O_4)O(C_6H_{10}O_4)OH$	CH_3	CH_3		α	A		580 [Z]	562	566 [X]
CH_3	$CH_2C_6H_5$				C		581	563	567
$CH(CH_3)_2$	$CH_2CH_2CH(CH_3)_2$				C		589	589	575
$CH(CH_3)_2$	$(CH_2)_2SCH_3$				C		593	575	579
$CH(CH_3)_2$	$CH_2C_6H_5$				D		593	575	579
CH_2CH_3	$CH_2C_6H_5$				C		595	577	581
$CH(CH_3)_2$	$CH_2C_6H_5$				C		609	591	595

n.a., not applicable; DB, # additional double bonds. (cf. Figure 7); str, structure A–D in Figure 7.
Compounds arranged in order of increasing molecular weight. Boxed, shaded section refers to m.w. range of interest in defining unknown 5-min RT compound.
[1]C_{10}-H in beta position; [2]$R_1 \leftrightarrow R_2$ isomer; [3]+ R_3 stereoisomer.

and valine with the known 5′-substituents valine, phenylalanine, isoleucine or leucine. However, unusual recombinations within this limited subset did not allow satisfactory accommodation of the 570 m.w., particularly its even value. Consideration of the twenty major genetic code-encoded amino acids was particularly driven by the need to introduce nitrogenous R-group containing amino acids such as lysine to provide an even N-number. This consideration is not necessarily unlikely since new amino acids have been discovered in ergot alkaloid structures, including methionine and norleucine (Cvak et al., 1996, 2005), although apparently not yet the required lysine, asparagine, tryptophan or glutamine, each capable of introducing a single N-atom, or arginine capable of introducing three N-atoms. The most acceptable posited ergopeptine structure starts with an m.w. of 572 in Table 3 and would include dehydrated 2′-ala/5′-arg, 2′-val/5′gln, 2′-val/5′-lys, 2′-gln/5′-val, 2′-lys/5′-val, 2′-asn/5′-leu or 2′-asn/5′-ile combinations since one of each combination is in the already known amino acid subset and would require only one unusual amino acid. In addition, another double bond or even a ring would then be required to reduce the m.w. by 2 hydrogen atoms. An example structure from this category derived from the 2′-lys/5′-val combination is illustrated in Figure 8. It is unknown whether this structure would explain the uncharacteristic fragmentation (Figures 3 and 6), although it is strongly suspected that it should provide *m/z* 221 and 206 fragments similar to *m/z* 223/208 fragments generally seen from ergots bearing intact ergoline ring systems (Lehner et al., 2004a, 2005).

The structurally different posited chanoclavine-fructosides (Figure 10) derive from previous work of other investigators (Flieger et al., 1997). The *C. fusiformis* strain W1 was found capable of considerable fructosylation ability toward chanoclavine; however, in order to accommodate the 570 m.w. in our work, demethylation and reduction of two double bonds was necessary. The advantage of such a structure is that it avoids the even molecular weight problem inherent in the ergopeptines; it provides sufficiently high mass by incorporation of the fructofuranosyl-(2,1)-O-beta-D-fructofuranoside group; and it provides at least a possible explanation for the uncharacteristic fragmentation pattern of this compound by positing a secoergoline ring system. Fungal databases at the USDA Agricultural Research Service (http://nt.ars-grin.gov) indicate reported *Claviceps* infection of cenchrus, panicrum, pennisetum and sorghum species, thereby providing a possible pretext for infection of forage grasses or grains. Unfortunately, the structure as shown in Figure 10A changes the level of double bond conjugation in the lysergic ring system, which would likely dramatically change its fluorescence characteristics, in turn, and make it undetectable by this methodology. In addition, it

may occur through biochemical interactions between bacteria and fungal mycotoxins, as set forth by Cho et al. (2010), Meca et al. (2010) and Thibodeau et al. (2004), for example. Preliminary experiments suggest that the unfermented grasses alone do not contain the new presumed ergot alkaloid, although further experiments are required to demonstrate this definitively. Whether the compound is in fact a direct microbial byproduct or a fungal endophytic product metabolically induced or altered by fermentative microorganisms thus remains to be determined.

In conclusion, it is our hope that, despite the inherent difficulties of the proposed structures, they may provide useful conceptual starting points for eventual elucidation of this new presumed ergot alkaloid.

Declaration of interest

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