by nitrite is also demonstrated (Knowles et al., 1974; Kurechi et al., 1979, 1980b). Although I and II can undergo reaction with nitrite to suffer ortho nitrosation or ortho nitration, no such compounds were detected in the reaction mixtures of the present investigation.

The structural analysis of the reaction products of I and II with nitrite indicated that most of the reactions may be the addition of nitrite to the olefinic group of these compounds, giving the complicated reaction products. One of these complex reactions may be the addition reaction of nitrite to produce nitroso–nitro compounds, which might be readily transformed into its tautomer, oxime–nitro compounds, and subsequently decarboxylated and dehydrated into furoxan derivatives, I(A) and II(A). Nitrite has been shown to react with olefins such as sorbic acid to produce nitroso–nitro or its tautomeric form, the oxime–nitro compound, which might be dehydrated into the furoxan derivative as the closed ring structure (Osawa et al., 1979).

Formation of these complex products by reaction of I and II with nitrite must consume nitrite which would otherwise be available for the nitrosamine formation. Caffeic acid (III) and chlorogenic acid (IV) may suffer such kinds of reactions in their olefinic groups besides conversion into the corresponding quinones. The decrease in nitrite level and inhibition of nitrosamine formation by these diphenols may be attributed to the complex reaction of nitrite to the phenolics.

Although the formation of these complicated products by reaction of naturally occurring *p*-hydroxycinnamic acid derivatives with nitrite might retard formation of carcinogenic nitrosamines, carcinogenicity of the reaction products must be carefully investigated.

Registry No. I, 7400-08-0; I(B), 123-08-0; II, 1135-24-6; III, 331-39-5; IV, 327-97-9; nitrite, 14797-65-0.

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Quadrupole Mass Spectrometry/Mass Spectrometry of Ergot Cyclol Alkaloids

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The quadrupole MS/MS spectra of the ergot cyclol alkaloids are reported. The alkaloids were ionized by chemical ionization (CI) with isobutane as a reagent gas. Both parent and daughter experiments on major fragment ions in the isobutane CI spectra were used to differentiate all 12 ergot peptide alkaloids studied. Samples can be analyzed for these alkaloids with much less cleanup than required by other methods.

Considerable interest has been generated recently in using the rather new technique of tandem mass spectrometry or mass spectrometry/mass spectrometry (MS/MS) to analyze crude mixtures of organic compounds. This is particularly true when the compounds of interest are not amenable to separation by gas chromatography/mass spectrometry (GC/MS), because any mass spectrometric study of these compounds requires rigorous purification prior to mass spectrometric analysis. The approach of MS/MS is to utilize one stage of mass separation to isolate the compound of interest from the matrix and a second stage of mass separation for analysis. Usually

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a molecular ion (M^+), a protonated molecule ($M + H^+$), or a molecular anion ($M^-\cdot$) (depending on the ionization mode) is focused by the first mass filter and undergoes collisionally activated dissociation (CAD) by collision with a target gas, followed by analysis of the product ions in the second mass filter. The wide variety of emerging instrumentation being developed for MS/MS studies has been reported in several excellent reviews (Kondrat and Cooks, 1978; McLafferty, 1980).

The fungus *Claviceps purpurea* (Clavicipitaceae) products ergot alkaloids that have been associated with toxic syndromes ("ergotism") in domestic livestock grazed on pastures or grains infected with the fungus (Porter and Betowski, 1981). Ergot-contaminated feeds are known to have a severe economic impact on both grain utilization and livestock production (Porter and Betowski, 1981); however, the economic impact of chronic subclinical concentrations of these alkaloids on livestock production is unclear. The similarity of structure and properties of the ergopeptines or cyclol alkaloids makes them difficult to separate and analyze. Several EI and CI mass spectral studies of the cyclol alkaloids have been reported (Porter and Betowski, 1981; Barber et al., 1965; Vokoun and Rehaeck, 1975). However, some of the isomers give nearly identical CI and EI spectra, making differentiation of these isomers quite difficult. Also, the alkaloids as a class are not amenable to GC, so chromatographic cleanup of sample is necessary before mass spectrometric analysis. Recently, in a report dealing with HPLC/MS and *B/E*-linked scans of ergot clavine alkaloids (Eckers et al., 1982), LC/MS analysis of one of the cyclol alkaloids, ergokryptine, was reported. We have recorded quadrupole MS/MS spectra for 12 of the 14 known ergopeptine alkaloids (Bianchi et al., 1982) and report that all 12 can be differentiated by MS/MS experiments. The use of tandem mass spectrometry as an analysis procedure for these components allows their determination from a complex matrix with substantially less sample cleanup and purification.

EXPERIMENTAL SECTION

A Finnigan 4535/TSQ quadrupole mass spectrometer equipped with pulsed positive–negative ion chemical ionization was used in the positive and negative ion chemical ionization modes. Isobutane was used as the reagent gas (0.25 torr). Argon was used as the target gas in CAD experiments. The electron energy was 70 eV and the source temperature was 140 °C. Samples were introduced via the direct insertion probe, which was heated either by a ballistic ramp or by feedback control using the data systems GC control output to heat the probe. Data acquisition and mass spectrometer control were accomplished by an Incos 2300 data system that controlled scanning and mass setting parameters for the quadrupoles in MS/MS experiments. Mass spectra were recorded by operating quadrupole Q_1 and Q_2 in the all pass mode and scanning Q_3. Daughter experiments were carried out by setting a given m/z value to pass in Q_1, operating Q_2 in the all-pass mode at pressures in the range of (1–3) × 10^{-3} torr, and scanning Q_3 normally. In parent experiments, Q_1 was scanned while Q_3 was set to pass only the single daughter mass value.

The 12 alkaloids were obtained and checked for purity as previously reported (Porter and Betowski, 1981). The sample from *C. purpurea* infected barley was prepared as reported by Krieger (1982).

RESULTS AND DISCUSSION

The isobutane positive ion CI (PICI) mass spectra for the peptide alkaloids (Table I) are similar to those reported previously by Porter and Betowski except that protonated molecules ($M + H^+$) were observed in our study. The major fragmentation is cleaved at the bond joining the tricyclic peptide moiety with the lysergic acid amide portion of the molecule yielding the protonated ions AH^+ and BH^+ (Figure 1). Fragment BH^+ also undergoes loss of the R_1 group and two carbonyl groups to form CH^+. The variation of abundance of $(M + H)^+$ and of AH^+, BH^+, and CH^+ with time of elution from the probe (Figure 2) indicate that little decomposition of the sample occurs. Table II shows the isobutane negative ion CI (NICI) mass spectra of the 12 alkaloids. Molecular anions ($M^-\cdot$) are observed as small signals for all 12 alkaloids. The major ion observed in the spectra is the B^- fragment from the tricyclic peptide moiety. Small signals are also observed for $(A - H)^-$ and $(C - H)^-$. Figure 3 shows the positive and negative chemical ion spectra of ergostine for comparison. Spectra of pure samples of similar isomer pairs ergosine and β-ergosine, ergoptine and β-ergoptine, or α- and β-ergokryptine are virtually indistinguishable by mass spectrometry along in either the PICI or NICI mode. Furthermore, the isomer sets constituting the ergosines and ergonine or the ergoptines and ergocornine have the same molecular weight and A and B fragments and differ only in the rather small C fragments, which makes sorting out

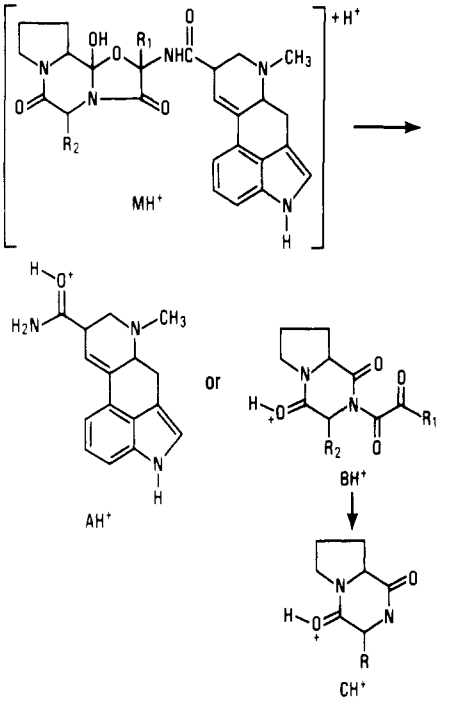

Figure 1

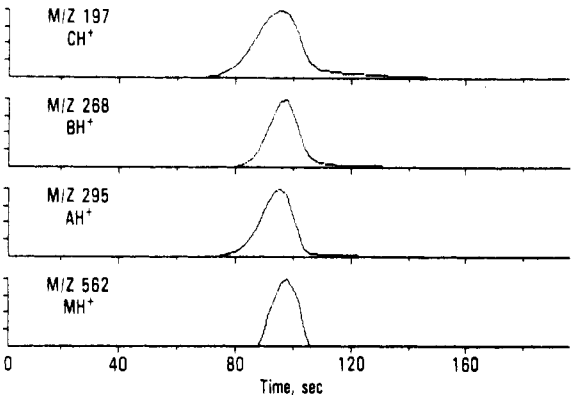

Figure 2. Ion profiles of $(M + H)^+$ (562), AH^+ (295), BH^+ (268), and CH^+ (197) for ergocornine vs. time (probe temperature).

Table I. Isobutane CI-MS of Ergot Peptide Alkaloids

		m/z (rel abundance)			
	R_2	$(M^+H)^+$ (× 10^{-2})	AH^+ [a]	BH^+	CH^+
ergotamine group ($R_1 = CH_3$)					
ergotamine	$PhCH_2$	582 (0.1)	268 (100)	315 (72)	245 (10)
ergosine	*i*-Bu	548 (10)	268 (100)	281 (52)	211 (10)
β-ergosine	*sec*-Bu	548 (5)	268 (100)	281 (76)	211 (11)
ergovaline	*i*-Pr	534 (8)	268 (100)	267 (74)	197 (12)
ergoxine group ($R_1 = C_2H_5$)					
ergostine	$PhCH_2$	596 (4)	268 (100)	329 (86)	245 (5)
ergoptine	*i*-Bu	562 (51)	268 (100)	295 (82)	211 (12)
β-ergoptine	*sec*-Bu	562 (18)	268 (100)	295 (72)	211 (11)
ergonine	*i*-Pr	548 (78)	268 (100)	281 (72)	197 (11)
ergotoxine group (R_1 = *i*-Pr)					
ergocrystine	$PhCH_2$	610 (0.1)	268 (100)	343 (84)	245 (12)
α-ergokryptine	*i*-Bu	576 (35)	268 (100)	309 (64)	211 (7)
β-ergokryptine	*sec*-Bu	576 (52)	268 (100)	309 (67)	211 (8)
ergocornine	*i*-Pr	562 (14)	268 (100)	297 (72)	197 (11)

[a] See Figure 1 for identity of fragments A, B, and C.

Table II. Isobutane Negative Ion CI-MS of Ergot Peptide Alkaloids

		m/z (rel abundance)			
	R_2	M^- (×10^{-2})	$(A - H)^-$	B^-	$(C - H)^-$
ergotamine group ($R_1 = CH_3$)					
ergotamine	$PhCH_2$	581 (2)	266 (0.5)	314 (100)	243 (1)
ergosine	*i*-Bu	547 (22)	266 (1)	280 (100)	209 (1)
β-ergosine	*sec*-Bu	547 (35)	266 (0.5)	280 (100)	209 (2)
ergovaline	*i*-Pr	533 (10)	266	266 (100)	195 (1.5)
ergoxine group ($R_1 = C_2H_5$)					
ergostine	$PhCH_2$	595 (43)	266	328 (100)	243 (0.3)
ergoptine	*i*-Bu	561 (13)	266	294 (100)	209 (0.3)
β-ergoptine	*sec*-Bu	561 (26)	266 (0.3)	294 (100)	209 (1)
ergonine	*i*-Pr	547 (85)	266 (0.5)	280 (100)	195 (0.5)
ergotoxine group (R_1 = *i*-Pr)					
ergocristine	$PhCH_2$	609 (12)	266	342 (100)	243 (0.2)
α-ergokryptine	*i*-Bu	575 (65)	266 (0.1)	308 (100)	209 (0.1)
β-ergokryptine	*sec*-Bu	575 (37)	266 (0.1)	308 (100)	209 (0.2)
ergocornine	*i*-Pr	561 (49)	266 (0.1)	294 (100)	195 (0.2)

Table III. Daughters of Negative Ion B^- Fragments for the Ergot Peptide Alkaloids

				daughters m/z (rel intensity)	
	R_2	M_r	B^-	$(C - H)^-$ [a]	other
ergotamine group ($R_1 = CH_3$)					
ergotamine	$PhCH_2$	581	314 (15)	243 (100)	227 (1) 183 (3) 152 (6)
ergosine	*i*-Bu	547	280 (3)	209 (100)	237 (5) [B − 43], 152 (4)
β-ergosine	*sec*-Bu	547	280 (3)	209 (100)	265 (1) [B − 15], 259 (2) [B − 29], 152 (10)
ergovaline	*i*-Pr	533	266 (9)	195 (100)	251 (1) [B − 15], 152 (4)
ergoxine group ($R_1 = C_2H_5$)					
ergostine	$PhCH_2$	595	328 (10)	243 (100)	227 (1), 197 (5), 152 (6)
ergoptine	*i*-Bu	561	294 (10)	209 (100)	251 (5) [B − 43], 152 (4)
β-ergotine	*sec*-Bu	561	294 (11)	209 (100)	279 (1) [B − 15], 259 (2) [B − 29], 152 (3)
ergonine	*i*-Pr	547	280 (9)	195 (100)	265 (1) [B − 15], 152 (5)
ergotoxine group (R_1 = *i*-Pr)					
ergocristine	$PhCH_2$	609	342 (18)	243 (100)	227 (3), 211 (6), 152 (4)
α-ergokryptine	*i*-Bu	575	308 (4)	209 (100)	265 (7) [B − 43], 152 (2)
β-ergokryptine	*sec*-Bu	575	308 (5)	209 (100)	293 (1) [B − 15], 279 (2) [B − 29], 152 (4)
ergocornine	*i*-Pr	561	294 (17)	195 (100)	279 (1) [B − 15], 152 (4)

[a] Loss of R_1 + 2CO from B^-.

mixtures of these isomers difficult.

By contrast, MS/MS daughters of the B^- fragment in NICI yield clearly unique spectra for all 12 peptide alkaloids (Table III). Figure 4 shows the B^- daughter spectra for the three sets of isomers that are not distinguishable by single-stage mass spectrometry. In all cases, the base peak in the B^- daughter spectra is the $(C - H)^-$ ion. A small peak at m/z 152 is observed in all of the B^- daughter spectra, which presumably results from the direct loss of $R_2 + R_1$–CO–CO from B^-. When R^2 is an isobutyl group, B^- loses 43 u. When R_2 is *sec*-butyl, $(B - 43)^-$ is not observed but ions are observed at both $(B - 15)^-$ and $(B - 29)^-$. Both of these ion signals are absent from the spectra of the isobutyl isomers, making discrimination of all the isomers possible. The ergosines are easily differentiated from ergonine by the MS/MS daughters of the B^- fragment; the base peak in the daughter spectrum $(C - H)^-$ is at m/z 209 in the ergosines, whereas it is at m/z 195 in the ergonine. Similarly, the B^- daughters of the ergoptines yield a base peak $(C - H)^-$ of m/z 209, whereas in ergocornine it is at m/z 195. In single-stage mass spectrometry, the presence of several alkaloids is difficult to determine because of overlaps in the masses of various B and C fragments. For example, detection of ergosine or β-ergosine

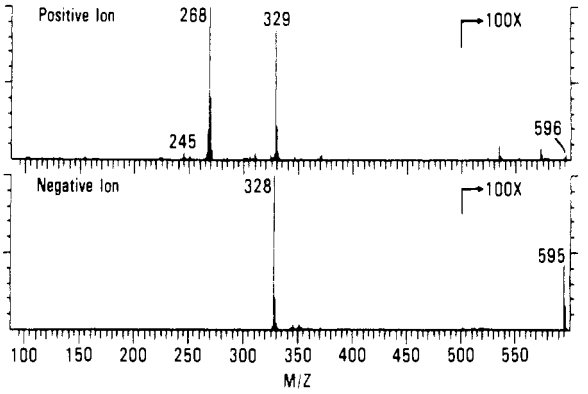

Figure 3. Positive ion (isobutane) chemical ionization and negative ion chemical ionization mass spectra of ergostine.

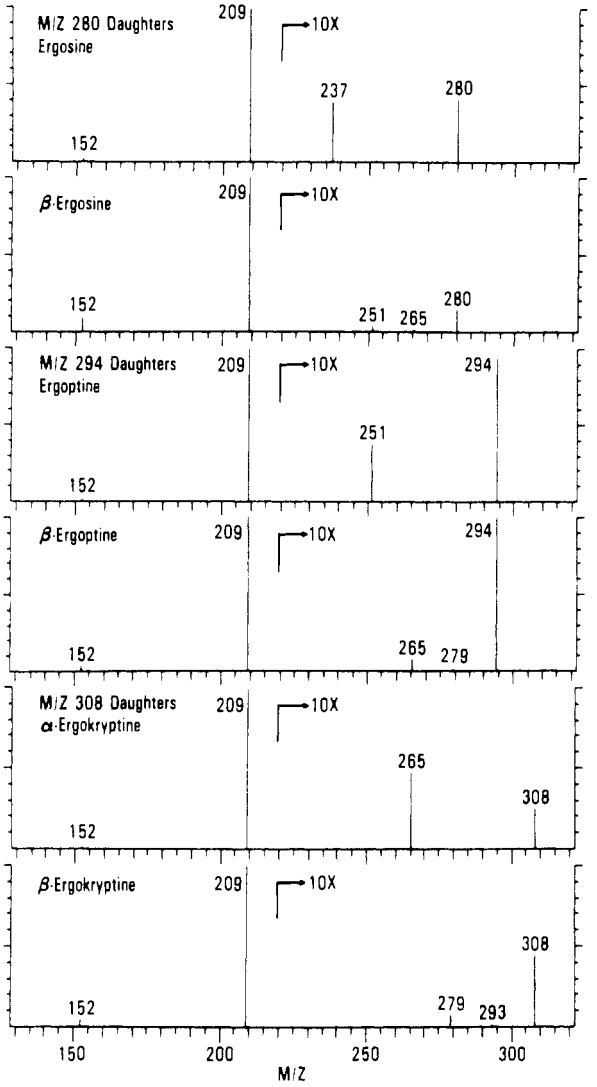

Figure 4. MS/MS daughters of NICI/MS B^- fragments for ergot peptide alkaloids.

in the presence of both ergonine and either or both of the ergoptines would be difficult by mass spectrometry because the ions characteristic of ergosines (at m/z 548, 268, 281, and 211 in PICI-MS and m/z 547, 266, 280, and 209 in NICI-MS) are present in the matrix of ergoptines and ergonine. By contrast, in MS/MS daughter ion experiments the ergosines are clearly separated from interference with the ergoptines since the B^- fragments are different (m/z 280 and m/z 294, respectively), and sequential scanning of the expected B^- fragments can reveal unambiguously which alkaloids are present in the matrix.

Because of the low abundance of $M + H^+$ in PICI-MS and $M^{-\cdot}$ in NICI-MS, no daughter experiments from the protonated molecular ion or anion were attempted. Parent experiments, in which Q_1 was scanned while Q_3 remained fixed at the B^- fragment in NICI-MS or the $(AH)^+$ or $(BH)^+$ fragment in PICI-MS, yielded small signals at $M^{-\cdot}$ and $M + H^+$, respectively, demonstrating that the alkaloids were being volatilized intact and that the fragments analyzed did indeed come from the alkaloid molecules.

Eckers et al. (1982) concluded from their linked B/E scan studies of ergot clavine alkaloids that the use of MS/MS alone for the study presented problems. They reported that isomeric mixtures were difficult to resolve and that interferences of fragment ions of other clavine alkaloids caused problems. With the peptide alkaloids this is not true. We have shown that mixtures of isomeric peptide alkaloids can be differentiated by quadrupole MS/MS. All interfering fragments in CI-MS of mixtures of these alkaloids were resolved by careful selection of MS/MS experiments.

Analysis of a crude extract of sclerotia (*C. purpurea*) isolated from barley associated with lameness, agalactica, and poor performance in swine (Krieger, 1982) demonstrated that MS/MS can be used to identify ergot peptide alkaloids directly from a crude extract. Previous investigations of this extract by conventional means (i.e., preparative TLC of crude extracts followed by cochromatography of the isolated fractions in several solvent systems with authentic standards and ultraviolet analyses and low-resolution electron impact mass spectroscopy on fractions isolated from the preparative TLC plates) showed the sclerotia to contain chanoclavine, ergonovine, ergocristine, ergocornine, and ergotamine (Krieger, 1982). Several other minor alkaloids were detected, but their identification could not be unequivocally made owing to the small amount of materials extracted. The crude extract was analyzed by direct insertion probe in the NICI-MS mode. On successive scans the seven B^- fragments for the known peptide alkaloids were focused by Q_1, and the CAD daughter spectra were recorded. The daughter spectra generated by these experiments were then compared to the daughter spectra generated by analysis of the pure standards. The spectrum of m/z 342 daughter ion from the crude extract was identical with the m/z 342 daugther spectrum of ergocristine. The large response of daughter signal at m/z 342 indicated ergocristine was the most abundant peptide alkaloid in the matrix. Similarly, the large signal observed for m/z 314 daughters (20% as intense as for m/z 342 daughters) and the identity of the daughter spectrum with that of the standard ergotamine indicated ergotamine was also a major peptide alkaloid in the sample. A much smaller signal was observed for m/z 328 daughters (about 2% as intense as for m/z 342 daughters). The m/z 328 daughter spectrum from the sample was very similar to the m/z 328 daughter spectrum of pure ergostine. All m/z 328 daughter fragments of the standard were observed at the same relative intensity ratios in the daughter spectrum from the matrix. Additional daughters of moderate abundance at m/z 271, 285, and 313 indicated that some of the m/z 328 ions in the matrix arose from a component or components other than ergostine. The daughter spectrum of the m/z 308 ion was an intense signal observed from the contaminated barley matrix (17% as intense as the m/z 342 daughter signal). This daughter spectrum was identical with a composite daughter spectrum of the m/z 308 ion from pure α-ergokryptine and β-ergokryptine. Based on the relative areas of the daughter at m/z 265 compared to the daughters of m/z 279 and 293,

the ratio of α-ergokryptine to β-ergokryptine was around 5 to 1. The other large signal observed in the sample extract was for m/z 294 daughters (22% as intense as m/z 342 daughters). The m/z 294 daughter spectrum had a m/z 195 ion as the base peak with a small peak (~2% of that of m/z 195) at m/z 209, indicating ergocornine was the major peptide alkaloid shown by the m/z 294 daughters from the sclerotia extract matrix. Because the daughter signals for the ergoptine and β-ergoptine were overshadowed by daughter signals from ergocornine (50 to 1 concentration ratio), the presence of ergoptine isomers was noted; however, the determination of which ones were present could not be made.

Analysis of the small signal observed for m/z 280 daughters from the sample (~3% as intense as for m/z 342 daughters) revealed the presence of ergosine isomers and ergoine. The base peak in the m/z 280 daughters was at m/z 209 (100), with smaller peaks observed at m/z 195 (7) and m/z 181 (3). The m/z 209 base peak in the m/z 208 daughter spectrum indicated that ergosine and/or β-ergosine were present in the matrix. Both compounds probably were present, as indicated by daughter signals at m/z 237 (ergosine) and at m/z 265 and m/z 259 (β-ergosine). The signal at m/z 195 indicated the presence of a smaller amount of ergonine, whereas the small signal at m/z 181 suggested ergobutyrine, although no standard for ergobutyrine was available. A small signal was observed for m/z 266 daughters (~1% as intense as the m/z 342 daughter signal). This signal was clearly observable above the system noise; the m/z 266 daughter spectrum from the barley matrix was identical with the m/z 266 daughter spectrum for ergovaline, indicating its presence in the sample. Thus, the presence of at least 10 of the 14 peptide alkaloids could be demonstrated by MS/MS in a crude extract of a sample suspected to contain ergot alkaloids. MS/MS in a single analysis has rapidly identified the peptide alkaloids from a simple extract. The detection and identification of these compounds by MS/MS were on approximately 1 μg of crude alkaloid material (colorimetrically determined by using p-(dimethylamino)benzaldehyde) and amply demonstrates the applicability of this technique for the analyses of the individual ergot peptide alkoids without the rigorous cleanup required by current procedures.

Registry No. Ergotamine, 113-15-5; ergosine, 561-94-4; β-ergosine, 60192-59-8; ergovaline, 2873-38-3; ergostine, 2854-38-8; ergoptine, 29475-05-6; β-ergoptine, 65756-55-0; ergonine, 29537-61-9; ergocrystine, 511-08-0; α-ergokryptine, 511-09-1; β-ergokryptine, 20315-46-2; ergocornine, 564-36-3.

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Determination of Cyanogenic Compounds by Thin-Layer Chromatography. 1. A Densitometric Method for Quantification of Cyanogenic Glycosides, Employing Enzyme Preparations (β-Glucuronidase) from *Helix pomatia* and Picrate-Impregnated Ion-Exchange Sheets

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A densitometric method for the quantitative determination of cyanogenic glycosides is described. The method is based on the release of HCN catalyzed by the enzyme preparation β-glucuronidase from *Helix pomatia* and subsequent direct detection of HCN on hydrophobic, picrate-impregnated, transparent, ion-exchange sheets. The sheets are placed directly on the enzyme-wetted chromatogram, and the intensities of the obtained spots are determined. No significant changes in intensities of spots occur over a period of 28 days, if the sheets are protected from corrosive vapors. If a densitometer is not available, or when a rapid field test is required, a semiquantitative determination is possible by visual inspection. The method was found suitable for the separate estimation of cyanogenic principles in cassava meal, lima beans, and linseed meal.

The presence of cyanogenic constituents, i.e., glycosides, cyanogenic lipids, and cyanhydrins in food and fodder of plant origin, is a severe problem in many parts of the developing countries. Especially the occurrence of considerable amounts of linamarin and lotaustralin in *Manihot esculenta* Crantz (cassava) is troublesome, since this plant and its products form one of the most important sources of dietary carbohydrate for millions of people (Phillips, 1977; Nartey, 1978). Similarly, *Phaseolus lunatus* L. (lima bean), an important edible legume especially grown in South and Latin America, contains linamarin and lotaustralin (Conn, 1979), while the major cyanogenic constituents in the widely used fodder product linseed

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