AMINO ACIDS IN SOUND AND ERGOT-INFECTED CEREALS AND GRASSES[1]

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ABSTRACT

Acid hydrolysates of paired samples of ergot bodies [sclerotia of *Claviceps purpurea* (Fr.) Tul.] and sound kernels of 11 cereal grains and grasses were analyzed. Each pair contained sclerotia and normal kernels collected from the same plant. The ergot bodies contained, in addition to known amino acids, at least five unidentified ninhydrin-positive compounds. Amount of the major unidentified compound or compounds ranged from trace to 1.37 g. and averaged 0.53 g. per 100 g. total ninhydrin-positive compounds. Kjeldahl-nitrogen was higher in ergot bodies than in sound kernels. Proteins of the sclerotia contained more threonine, lysine, isoleucine, valine, alanine, serine, aspartic acid, and methionine; and less proline, glutamic acid, and cystine than proteins of sound kernels. It is possible that increased amounts of amino acids in sclerotia may be due, in part, to unknown compounds eluted in the same positions as the identified amino acids.

Protein changes in diseased plants have been the subject of many investigations (1). The effect of ergot is of particular significance because ergot bodies actually replace kernels in spikes (heads) or panicles that are infected.

Ergot is a disease of cereal crops and many grasses, caused by the fungus *Claviceps purpurea* (Fr.) Tul. Infection occurs in the florets (flowers) at the time of blossoming, and purplish-black sclerotia form in place of kernels. Sclerotia or ergot bodies contain alkaloids which are toxic to man and animals causing grave concern over ergoty grain or hay. Many sclerotia are nearly the same size and shape as normal kernels and are difficult to remove from grain. Fortunately, the ergot bodies are lower in specific gravity and thus can be separated by floating. Damage to cereal grains by ergot can be of major significance to the farmer and to the U.S. economy. Grain harvested from an infected field has ergot bodies mixed with the kernels. Both yield and quality of grain are affected adversely.

Ergot occurs principally in cross-pollinated cereal crops or grasses—those in which florets tend to remain open for a longer than "normal" period, and in crops in which sterility occurs. Rye is notorious for harboring ergot. It is cross-pollinated, has large florets which remain open usually until pollinated, and has a high degree of natural sterility. Ergot occurs in self-pollinated species such as wheat, oats, and barley but to a lesser degree than in rye. Infection in self-pollinated species usually is noted only when weather conditions are optimum for infection. If hybrid wheat becomes an important crop, ergot could become a more serious problem in wheat. Because florets of the cytoplasmic male sterile plants used in hybrid wheat production generally remain open until pollinated from some other source, opportunity for infection by ergot is greatly enhanced.

Readily available and reliable methods for determining the presence of ergot-damaged cereals, especially in ground products, require time-consuming and

[1]Cooperative investigations between the Agricultural Research Service, North Central Region, U.S. Department of Agriculture, and College of Agricultural and Life Sciences, University of Wisconsin, Madison.

Mention of specific instruments or trade names is made for identification purposes only and does not imply any endorsement by the U.S. government.

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expensive biological assays. According to Bove (2), 20 known amino acids and amines and several unidentified nitrogen-containing compounds were found in ergot sclerotia. Little information is available on quantitative differences in amino acid composition and other nitrogen-containing compounds in sound kernels and in ergot sclerotia. Such differences are the subject of this preliminary investigation.

MATERIALS AND METHODS

Materials

Ergot sclerotia and healthy kernels were separated by hand from 11 samples of cereals or grasses collected in 1971. They were three samples of rye and one sample each of triticale, wheat, oats, barley, *Agropyron inerme, Elymus cinereus, Bromus marginatus,* and an unidentified grass. The barley was a threshed sample from Bozeman, Mont. The other cereals were collected as spikes or panicles at the Idaho Agricultural Experiment Station, Aberdeen. The grass samples were collected along roadsides in southern Idaho.

Methods

Moisture and Kjeldahl nitrogen were determined according to the American Society of Brewing Chemists methods of analysis (3). Kjeldahl nitrogen data are reported on a moisture-free basis. Replicated amino acid analyses were performed on a Beckman 121 automatic amino acid analyzer. Details of acid hydrolysis, assay, and data processing were described by Pomeranz and Robbins (4). Amino acid values are presented in this report as g. amino acid per 100 g. amino acid recovered. Average recoveries (based on Kjeldahl nitrogen contents) were 89.8% for the sound kernels and 78.2% for the ergot sclerotia, indicating relatively large amounts of nonprotein nitrogenous compounds in the latter. Recoveries for kernels from the grasses were lower than for kernels from the cereals, especially when compared to wheat, rye, and triticale, which are hull-less.

RESULTS AND DISCUSSION

A typical amino acid chromatogram of an acid hydrolysate of ergot sclerotia from barley is given in Fig. 1. At least five unidentified peaks were present in addition to 18 amino acids and ammonia. The concentrations of amino acids, ammonia, and the five unidentified nitrogenous compounds varied. All sclerotia samples, but none of the sound kernels, contained the five unidentified compounds. Lysine was preceded by an irregular peak (I in Fig. 1). Peaks I and II were different from the acidic and neutral components which are a normal feature of the amino acids resolved on the short column. A large well-resolved peak (III) was recorded immediately preceding arginine. On the long column there was a trailing shoulder (IV) on the valine peak between valine and methionine. Finally a low peak (V) was recorded between the norleucine standard and tyrosine.

The presence of hydroxyproline might be of interest in view of the current interest in the part this amino acid plays in the structural and protective parts of the plant. Hydroxyproline would be eluted between aspartic acid and methionine sulfone. No measurable amounts of hydroxyproline were observed.

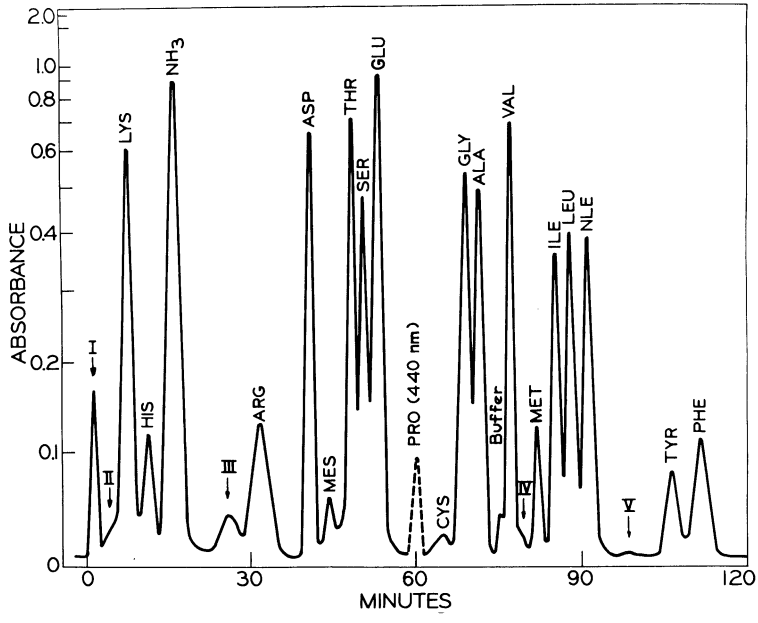

Fig. 1. Amino acid chromatogram of an acid hydrolysate of ergot sclerotia from barley.

Maximum, minimum, and mean values, and coefficients of variation for Kjeldahl nitrogen, 17 amino acids, and ammonia in acid hydrolysates of 11 pairs of samples are compared in Table I. Each pair was comprised of sound kernels and ergot sclerotia. A comparison of mean values indicates that ergot sclerotia contained on the average 1.55 times as much Kjeldahl nitrogen as sound grain. Amino acid composition of proteins in sound grain and ergot sclerotia differed. The ratios of concentrations of amino acids in proteins of sclerotia to concentrations of those amino acids in proteins of sound grains are given in Table II. It is possible, however, that increased amounts of amino acids in the sclerotia may be due, in part, to unknowns eluted in the same positions as the identified amino acids.

Simple correlation coefficients for the 11 pairs of samples indicate some interesting relationships (Table III). Kjeldahl nitrogen in both sound kernels and ergot sclerotia showed a negative association with lysine, glycine, alanine, valine, and isoleucine, and a positive relation with glutamic acid and cystine. The simple correlations between hydroxyamino acids and protein were negative. Significant correlations between Kjeldahl nitrogen and serine or tyrosine were recorded only for ergot sclerotia, however, only sound kernels showed a significant correlation between protein and threonine. Correlation coefficients for both the sound kernels and sclerotia had the same sign in all comparisons in which both coefficients were significant.

As shown in Fig. 1, peak III eluted immediately prior to arginine and was the largest component among the unidentified ninhydrin-positive compounds. Approximate amounts of this component, based on the contents of peak III (calculated as leucine), in the various species are given in Table IV. All ergot

TABLE I
Kjeldahl Nitrogen (%) and Amino Acid Composition[a] in Eleven Paired Samples of Sound Cereal or Grass Kernels and Ergot Sclerotia

	Sound Kernels				Ergot Sclerotia			
	Max.	Min.	Mean	Coeff. var.	Max.	Min.	Mean	Coeff. var.
Kjeldahl nitrogen	3.49	1.46	2.47	26.2	5.08	2.45	3.81	19.6
Lysine (lys)	5.6	2.6	3.7	22.8	10.1	7.1	8.8	9.0
Histidine (his)	2.4	2.0	2.2	4.3	2.9	2.2	2.5	8.6
Ammonia (NH_3)	4.0	2.8	3.5	9.1	4.0	2.9	3.4	10.7
Arginine (arg)	6.6	3.8	4.8	16.1	5.6	4.6	5.2	7.2
Aspartic acid (asp)	9.9	4.9	7.1	18.5	9.4	7.5	8.5	6.6
Threonine (thr)	4.6	2.4	3.2	20.2	8.8	7.0	8.0	6.9
Serine (ser)	4.8	3.4	3.9	11.0	5.2	4.0	4.7	8.5
Glutamic acid (glu)	33.4	19.2	27.1	14.7	23.0	10.8	16.6	21.6
Proline (pro)	15.0	6.4	10.5	24.8	7.5	4.6	5.8	15.2
Cystine (cys)	1.6	0.3	1.1	33.3	1.0	0.5	0.8	17.8
Glycine (gly)	6.0	3.6	4.4	15.8	5.3	3.9	4.6	7.9
Alanine (ala)	5.8	3.3	4.2	17.7	5.9	4.9	5.3	6.2
Valine (val)	5.9	4.2	4.8	11.5	6.8	5.4	6.1	6.7
Methionine (met)	2.9	2.2	2.5	9.0	3.7	2.5	3.0	13.1
Isoleucine (ile)	4.1	3.3	3.6	7.8	5.4	3.9	4.6	8.8
Leucine (leu)	7.6	5.7	6.3	8.6	6.7	4.8	5.6	10.8
Tyrosine (tyr)	3.2	1.7	2.3	19.5	3.0	2.4	2.7	6.4
Phenylalanine (phe)	5.5	4.3	4.7	7.7	4.4	3.5	3.9	7.2

[a]g. amino acid per 100 g. amino acid recovered.

TABLE II
Ratios of Certain Amino Acids in Proteins of Ergot Sclerotia and of Sound Grains

Amino Acid	Ratio
Threonine	2.50
Lysine	2.38
Isoleucine	1.28
Valine	1.27
Alanine	1.26
Serine	1.21
Aspartic acid	1.20
Methionine	1.20
Tyrosine	1.17
Histidine	1.14
Arginine	1.08
Glycine	1.05
Proline	0.55
Glutamic acid	0.61
Cystine	0.73
Phenylalanine	0.83
Leucine	0.89

TABLE III
Comparison of Significant[a] Correlation Coefficients for Components in Sound Cereal or Grass Kernels and Ergot Sclerotia[b]

		Kjeldahl N	Lys	His	NH_3	Arg	Asp	Thr	Ser	Glu	Pro	Cys	Gly	Ala	Val	Met	Ile	Leu	Tyr	Phe
Lys	S	−0.768																		
	E	−0.610																		
His	S																			
	E		0.735																	
NH_3	S																			
	E	0.651	−0.641																	
Arg	S				−0.615															
	E																			
Asp	S		0.884																	
	E		0.672		−0.644															
Thr	S	−0.867	0.973				0.799													
	E				−0.722															
Ser	S		0.698	0.694			0.638	0.744												
	E	−0.753	0.681		−0.681		0.751													
Glu	S	0.634	−0.914				−0.893	−0.868	−0.639											
	E	0.811	−0.801		0.843		−0.853		−0.908											
Pro	S		−0.755	−0.650		−0.706	−0.774	−0.741	−0.654	0.776										
	E		−0.756			−0.650	−0.646													
Cys	S	0.671			−0.876			−0.631												
	E	0.661																		
Gly	S	−0.816	0.961	0.637			0.839	0.977	0.814	−0.868	−0.807	−0.627								
	E	−0.727	0.734		−0.602		0.902		0.819	−0.904	−0.635									
Ala	S	−0.673	0.946	0.610			0.905	0.900	0.645	−0.978	−0.782		0.910							
	E	−0.620	0.704		−0.756		0.874		0.749	−0.888			0.791							

Val	S	−0.636	0.725	0.615			0.656	0.725		−0.878	−0.765		0.705	0.838										
	E	−0.947			−0.737				0.824	−0.867			0.749	0.723										
Met	S																							
	E	−0.788													0.751									
Ile	S	−0.716	0.786	0.688			0.690	0.823	0.636	−0.863	−0.850		0.816	0.844	0.951									
	E	−0.900	0.732		−0.773		0.761		0.779	−0.938			0.906	0.799	0.897	0.649								
Leu	S			0.672		0.670				−0.654	−0.748			0.648	0.836		0.793							
	E	−0.835	0.741		−0.756		0.857		0.882	−0.955		−0.620	0.919	0.906	0.892		0.928							
Tyr	S					0.790					−0.655					0.718								
	E	−0.688			−0.799		0.756		0.818	−0.863			0.683	0.835	0.772		0.764	0.835						
Phe	S																							
	E				−0.767		0.799		0.685	−0.800			0.653	0.898	0.697		0.712	0.829	0.923					

[a]Values of 0.602 and 0.735 and above (underlined) were significant at the 5 and 1% levels, respectively.
[b]Top and bottom row for sound kernels (S) and ergot sclerotia (E), respectively.

sclerotia contained the component(s) although concentrations varied widely. Amount of the unidentified component(s) in peak III ranged from trace to 1.37 g. (average 0.53 g.). Quantitative comparisons of the other unidentified peaks are not listed because of their relatively small amounts. It is not known whether the content of the unidentified components is related to the presence of any of the known alkaloids in ergot sclerotia. Kroeller (5) and Marine Font et al. (6) indicated that available methods for detection of ergot in cereal products are unsatisfactory, and that good chemical methods are needed to replace the reliable but rather complex biological methods.

TABLE IV
Relative Amounts of Unidentified Peak (III) in Ergot Sclerotia

Species	Unidentified Peak[a]
Wheat	0.50
Oats	0.92
Rye	1.37
Rye	0.65
Rye	0.78
Triticale	0.35
Unidentified grass	0.24
Agropyron inerme	0.29
Elymus cinereus	trace
Bromus marginatus	0.23

[a]g. per 100 g. ninhydrin-positive compounds, calculated as leucine.

Further studies of free amino acids and ninhydrin-positive compounds in sclerotia or in hydrolyzed fractions (i.e. aqueous or salt extract) of ergot sclerotia could provide useful information on changes in crude protein and could provide a basis for detection of ergot in cereal products. Examination by ion-exchange chromatography of the acid hydrolyzed and unhydrolyzed nonprotein fractions should be informative.

Acknowledgment

The technical assistance of J. T. Gilbertson is gratefully acknowledged.

Literature Cited

1. URITANI, I. Protein changes in diseased plants. Annu. Rev. Phytopathol. 9: 211 (1971).
2. BOVE, F. J. The story of ergot. J. Karger: Basel, Switzerland (1970).
3. AMERICAN SOCIETY OF BREWING CHEMISTS. Official methods of analysis. The Society: Madison, Wis. (1958).
4. POMERANZ, Y., and ROBBINS, G. S. Amino acid composition of maturing barley. Cereal Chem. 49: 560 (1972).
5. KROELLER, E. Ergot determination in cereal products. Deut. Lebensm. Rundsch. 66: 115 (1970) (Chem. Abstr. 73: 43334m, 1970).
6. MARINE FONT, A., MORENO MARTIN, F., and COSTES, C. Detection of ergot in flours. Circ. Farm. 30: 187 (1972) (Chem. Abstr. 78: 790t, 1973).

[Received April 4, 1974. Accepted June 24, 1974]