Registry No. *N*-Nitrosoproline, 7519-36-0; *N*-nitrososarcosine, 13256-22-9.

LITERATURE CITED

Bogovski, P.; Rooma, M.; Uibu, J.; Kann, J.; Tatus, O. *IARC Sci. Publ.* **1982**, *No. 41*, 259.
Eisenbrand, G.; Spiegelhalder, B.; Janzowski, C.; Kann, J.; Preussmann, R. *IARC Sci. Publ.* **1978**, *No. 19*, 311.
Fazio, T.; Havery, D. C.; Howard, J. W. *IARC Sci. Publ.* **1980**, *No. 31*, 419.
Fine, D. H.; Rieb, D.; Rufeh, F. *J. Chromatogr.* **1975**, *107*, 351.
Fine, D. H.; Rounbehler, D. P. *J. Chromatogr.* **1975**, *109*, 271.
Goff, E. U.; Fine, D. H. *Food Cosmet. Toxicol.* **1979**, *17*, 569.
Hotchkiss, J. H.; Libby, L. M.; Barbour, J. F.; Scanlan, R. A. *IARC Sci. Publ.* **1980**, *No. 31*, 361.
Kawabata, T.; Ishibashi, T.; Matsui, M. *Proc. Int. Congr. Food Sci. Technol., 4th* **1974**, *3*, 238.
Magee, P. N.; Montesano, R.; Preussmann, R. In "Chemical Carcinogens"; Searle, C. E., Ed.; Americal Chemical Society: Washington, DC, 1976; Mongr. No. 173, p 491.
Pensabene, J. W.; Feinberg, J. I.; Piotrowski, E. G.; Fiddler, W. *J. Food Sci.* **1979**, *44*, 1700.
Pollock, J. R. A. *J. Inst. Brew.* **1981**, *87*, 356.
Schlenk, H. *Anal. Chem.* **1960**, *32*, 1412.
Sen, N. P.; Donaldson, B. A.; Seaman, S.; Iyengar, J. R.; Miles, W. F. *IARC Sci. Publ.* **1978**, *No. 19*, 373.
Sen, N. P.; Seaman, S.; McPherson, M. *J. Food Saf.* **1980**, *2*, 13.
Sen, N. P.; Seaman, S.; Tessier, L. *IARC Sci. Publ.* **1982**, *No. 41*, 185.
Spiegelhalder, B.; Eisenbrand, G.; Preussmann, R. *IARC Sci. Publ.* **1980**, *No. 31*, 467.

Received for review December 29, 1982. Revised manuscript received May 18, 1983. Accepted June 6, 1983.

Ergosterol, Ergosta-4,6,8(14),22-tetraen-3-one, Ergosterol Peroxide, and Chitin in Ergoty Barley, Rye, and Other Grasses

Larry M. Seitz* and Yeshajahu Pomeranz

Ergosterol, ergosta-4,6,8(14),22-tetraen-3-one (ETO), ergosterol peroxide (EP), and chitin contents in ergot sclerotia ranged from 204 to 827, 0.74 to 5.6, 41 to 152, and 8210 to 9020 μg/g, respectively, in barley, rye, and five grasses. Ergosterol and chitin contents of ergot sclerotia were high compared to those of ergot-free grain, and removal of ergot sclerotia from grain kernels leaves behind only background levels of ergosterol. Ergot contamination increased the ergosterol content significantly more than the chitin content of the grain. Maximum ergot contamination allowed by U.S. Grain Standards is 0.1% by weight in some grains (barley and oats) and 0.3% in others (rye and wheat). Only at or above the 0.3% allowed ergot level would increases in ergosterol contents become significant. Ergosterol, ETO, EP, and chitin are components of other fungi besides *Claviceps* spp.

Ergot sclerotia are formed in place of normal seeds during parasitic attack of cereals and grasses by *Claviceps* spp., mainly *Claviceps purpurea* (Bove', 1970; Lorenz, 1979). Because ergot sclerotia usually contain toxic alkaloids, their presence is highly undesirable in grain used for food or feed. Although ergot is noticeable in the heads of cereal grains and in harvested whole grain, its visibility is greatly reduced or completely lost after the grain is ground and processed into food or feed. Methods for detecting ergot in cereal grains or their products include various color tests, pigment determinations, microscopic examination for ergot fragments, tests for toxicity to experimental animals (not often used), and chemical determinations of ergot alkaloids (Lorenz, 1979). High-pressure liquid chromatography is particularly useful for detecting specific alkaloids, especially those that are physiologically active (Scott and Lawrence, 1980; Young, 1981).

Assays for ergosterol (Seitz et al., 1977, 1979) and chitin (Donald and Mirocha, 1977) have been proposed for measurement of fungal invasion in grains. Studies of those assays, however, did not include samples of ergoty grains or seeds. Therefore, we measured ergosterol and chitin content of ergoty and ergot-free grains and grass seeds to determine whether either assay would be useful for detecting ergot contamination and to what extent assays of ergosterol and chitin would be affected by the presence of ergoty grain.

U.S. Grain Marketing Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Manhattan, Kansas 66502.

It has been know for a long time that ergosterol is a constituent of ergot (Tanret, 1889; Hart and Heyl, 1930), but quantitative information concerning its content in sclerotia or ergoty grains has been lacking. To the best of our knowledge little published information is available on the ergosta-4,6,8(14),22-tetraen-3-one (ETO) and ergosterol peroxide (EP) contents of cereals.

EXPERIMENTAL SECTION

Samples and Reference Compounds. Origins of samples of ergoty barley (*Hordeum vugare* L.), rye (*Secale cereale* L.), bromegrass (*Bromus sp.*), slender wheatgrass (*Agropyron sp.*), and an unidentified grass seed are given in Table I. Samples collected before 1978 were described by Pomeranz et al. (1975). Samples labeled "ergot free" in Table I represent grain or seed that remained after sclerotia were removed by hand.

Four commercial, ergot-free samples of barley (Table II) were obtained from the Federal Grain Inspection Service. The samples were selected to show a typical range in quality based on germination percentages and mold damage. Type of mold damage was not characterized.

Glucosamine and ergosterol were from Eastman Kodak Co. The latter was recrystallized twice from absolute ethanol. Authentic ETO and EP were prepared from ergosterol by the methods of Elks (1954) and White et al. (1973), respectively.

Assays. Ergosterol, ETO, and EP were extracted from ergot sclerotia, grains, and grass seeds as described previously (Seitz et al., 1979; Seitz and Paukstelis, 1977). Ergosterol and ETO were determined by a high-pressure liquid chromatography (HPLC) system consisting of a

Table I. Ergosterol, Ergosta-4,6,8(14),22-tetraen-3-one (ETO), and Ergosterol Peroxide (EP) Contents of Ergoty Barley, Rye, and Grass Seed

	ergosterol, μg/g		ETO, μg/g		EP, μg/g	
	sclerotia	sclerotia free	sclerotia	sclerotia free	sclerotia	sclerotia free
barley[a]	498	NS[h]	2.7	NS	62	NS
barley[b]	725	NS	NA[k]	NS	NA	NS
barley[c]	680	5.3[i]	4.7	tr[l]	41	NA
rye[d,e]	539	1.8[i]	5.6	tr	152	NA
rye[d,f]	544	NS	2.1	NS	80	NS
bromegrass[d,e]	478	NS	0.74	NS	NS	NS
bromegrass[g]	204	71.8[j]	2.0	0.63	67	10
slender wheatgrass[g]	448	31.2[j]	3.7	0.32	80	5
slender wheatgrass[g]	827	NS	2.1	NS	85	NS
unidentified grass[g]	400	27.3[i]	4.4	0.25	76	4

[a] 1975 crop, Montana. [b] 1978 crop, Montana. [c] 1971 crop, Montana. [d] Year unknown. [e] From central South Dakota. [f] From North Dakota. [g] From western North Dakota, 1974. [h] NS = no sample available. [i] Remaining sample after ergot sclerotia were removed. [j] After obvious sclerotia were removed, some seeds that remained showed various degrees of discoloration. [k] NA = sample available but not analyzed. [l] tr = trace.

Table II. Ergosterol and Chitin Contents of Ergot-Free Barley and Ergot Sclerotia from Barley

sample	germination, %	ergosterol, μg/g	chitin, μg/g
ergot-free 1	98	0.83	328
ergot-free 2	74	1.74	445
ergot-free 3	20	2.46	464
ergot-free 4	4	9.36	626
sclerotia, Montana, 1975		498	8210
sclerotia, Montana, 1978		725	9020

μBondapak C_{18} column (Waters Associates, Inc., Milford, MA) and 5% water in methanol. At a 1.67 mL/min flow rate, ergosterol was eluted at 8.2 min and ETO at 6.7 min. Ergosterol and ETO were detected by light absorption at 282 and 348 nm, respectively.

Interferences and low light absorption by EP precluded using the HPLC system for EP analysis. Instead, EP was determined by thin-layer chromatography (TLC) using Brinkman SILG-HR-25 precoated plates (activated 1 h at 110 °C) developed in chloroform–acetone (95:5 v/v). Ergosterol peroxide (>0.6 μg) produced a grey spot at R_f 0.27 when the developed plate was sprayed with 50% H_2SO_4 and warmed. Concentrations (about 2 μg/g minimum) of EP in samples were determined by visual comparison of sample and standard spots on the same plate. The same TLC system was used for verification of ergosterol (R_f 0.44) and ETO (R_f 0.61) in the extracts.

Chitin was assayed by using an ion-exchange amino acid analyzer to measure glucosamine liberated from chitin by acid hydrolysis (Hubbard et al., 1979).

Germination tests were performed by the Kansas State Seed Laboratory, Topeka, KS, according to procedures outlined by the U.S. Department of Agriculture (1975). Percent germination was determined after 7 days of germination between blotters at 20 °C.

RESULTS AND DISCUSSION

Extracts of ergot sclerotia produced spots or bands on TLC having R_f values and colors that were identical with those of corresponding ergosterol, ETO, and EP standards. The TLC plates were viewed before and after sulfuric acid treatment and under visible and UV lights. When ergosterol, ETO, and EP bands were scraped from the developed plates and the silica was washed with ethanol, the resulting solutions gave UV spectra matching those of the respective reference standards.

Ergosterol, ETO, and EP contents of ergot sclerotia from grains were similar to those from grasses (Table I). Ergot sclerotia contained slightly more ergosterol and about the same ETO as was produced by *Alternaria alternata* growing on moist corn–rice (1:1) for 21 days (Seitz and Paukstelis, 1977). *Aspergillus flavus* growing on moist milled rice for 10 days produced ergosterol levels of about 500 μg/g (Seitz et al., 1979). Samples of hand-selected, rotted, discolored corn kernels contained about 200 μg/g ergosterol (Seitz et al., 1977).

Removal of sclerotia from ergoty barley and rye gave samples consisting of mostly sound kernels with low ergosterol contents (Table I) similar to those for commerical, ergot-free samples from FGIS (Table II). However, the ergot-free grass samples were considerably discolored and had much higher ergosterol contents than those of ergot-free barley or rye (Table I). The sclerotia-free grass seed also contained ETO and EP (Table I).

Our finding of ETO in ergot sclerotia is consistent with a previous report of ETO production by two strains of *Claviceps* spp. from toxic Bermuda grasses (Porter et al., 1975). At least 13 other fungal species representing several genera are known to produce ETO (Price and Worth, 1974; Seitz and Paukstelis, 1977). Also, ETO has been isolated from mold-infested wheat flour (Cooks et al., 1970) and tobacco leaves (Tancogne, 1977).

Ergosterol peroxide is known to occur in fungi (Turner, 1971; Bhat et al., 1979) so its presence in ergot sclerotia is not unexpected. Its authenticity as a metabolite of fungi has been questioned because photosensitizing pigments could catalyze conversion of ergosterol to its peroxide during analysis (Adam et al., 1967). However, Bates and Reid (1976) have shown that *Penicillium rubrum* and *Gibberella fujikuri* produced EP naturally by two distinct pathways, one of which is photochemical. It is not known how much EP, if any, might have been formed during analysis of the ergot sclerotia.

Ergosterol and chitin contents of ergot-free barley and sclerotia from barley are compared in Table II. Sclerotia from barley (Montana 1978 sample) contained 874-fold more ergosterol, but only 28-fold more chitin, than barley with the least mold invasion (sample 1). Thus, ergot contamination would be expected to cause a greater percentage increase in the ergosterol content than in the chitin content of grain.

According to U.S. Grain Standards (U.S. Department of Agriculture, Federal Grain Inspection Service, Inspection Division, 1978), the maximum amount of ergot allowed is 0.1% by weight in barley, oats, and triticale and 0.3% in wheat and rye. One of the reasons for the differences in allowed levels in various grains is that barley, oats, and triticale are consumed, largely, as feeds without precleaning. Wheat and rye are mainly consumed as foods

that undergo extensive cleaning and, generally, removal of extraneous material during milling. It has been recommended that ergot should not exceed 0.1% of the barley fed to animals (Young, 1979). When barley is contaminated with 0.1% ergot sclerotia containing 600 μg/g ergosterol and 8500 μg/g chitin, the ergot contribution to ergosterol and chitin contents of the grain would be only 0.60 and 8.5 μg/g, respectively. With typical barley such as samples 1–4 in Table II, ergot contamination of 0.1% would not significantly increase the chitin content and only slightly increase the ergosterol content. This is particularly so with low-quality (mold-damaged) grain like sample 4. With 0.3% contamination allowed in wheat and rye, ergosterol contributed by ergot could represent a significant portion of the ergosterol content of the grain. The chitin content, however, would still be only slightly affected by 0.3% ergot.

Sound grain generally contains less than 3.0 μg/g ergosterol and less than 450 μg/g chitin. Damage by fungi (field or storage) could increase ergosterol levels to 10 μg/g or more and chitin levels to 1000 μg/g or more. In severely damaged grain, ergosterol and chitin levels could be substantially higher and comparable to those in ergot sclerotia (Table II). Consequently, a small amount of toxic ergot sclerotia or severely mold damaged grain (possibly toxic) could increase the ergosterol and chitin levels of a grain sample.

In evaluating the soundness of grain on the basis of ergosterol or chitin contents, the relatively wide variations in contents of those components in either sound or damaged grain must be considered. One must also consider that some of the total fungal contamination measured by ergosterol and chitin assays could be ergot. Neither assay will specifically indicate the presence of ergot because ergosterol and chitin are components of many fungi. The presence or absence of ergot should be confirmed by using a specific test for ergot.

ACKNOWLEDGMENT

We thank H. E. Mohr and J. D. Hubbard for technical assistance.

Registry No. ETO, 19254-69-4; EP, 2061-64-5; ergosterol, 57-87-4; chitin, 1398-61-4.

LITERATURE CITED

Adam, H. K.; Campbell, I. M.; McCorkindale, N. J. *Nature (London)* **1967**, *216*, 397.
Bates, M. L.; Reid, W. W. *J. Chem. Soc., Chem. Commun.* **1976**, 44.
Bhat, H. K.; Qazi, G. N.; Chopra, C. L.; Dhar, K. L.; Atal, C. K. *J. Indian Chem. Soc.* **1979**, *56*, 934.
Bove', F. M. "The Story of Ergot"; J. Karger: Basel, Switzerland, 1970.
Cooks, A. G.; Daftary, R. D.; Pomeranz, Y. *J. Agric. Food Chem.* **1970**, *18*, 620.
Donald, W. W.; Mirocha, C. J. *Cereal Chem.* **1977**, *54*, 466.
Elks, J. *J. Chem. Soc.* **1954**, 468.
Hart, M. C.; Heyl, F. W. *J. Am. Chem. Soc.* **1930**, *52*, 2013.
Hubbard, J. D.; Seitz, L. M.; Mohr, H. E. *J. Food Sci.* **1979**, *44*, 1552.
Lorenz, K. *CRC Crit. Food Sci. Nutr.* **1979**, *11*, 311.
Pomeranz, Y.; Robbins, G. S.; Briggle, L. W. *Cereal Chem.* **1975**, *52*, 108.
Porter, J. K.; Bacon, C. W.; Robbins, J. D.; Higman, H. C. *J. Agric. Food Chem.* **1975**, *23*, 771.
Price, M. J.; Worth, G. K. *Aust. J. Chem.* **1974**, *27*, 2505.
Scott, P. M.; Lawrence, G. A. *J. Agric. Food Chem.* **1980**, *28*, 1258.
Seitz, L. M.; Mohr, H. E.; Burroughs, R.; Sauer, D. B. *Cereal Chem.* **1977**, *54*, 1207.
Seitz, L. M.; Paukstelis, J. V. *J. Agric. Food Chem.* **1977**, *25*, 838.
Seitz, L. M.; Sauer, D. B.; Burroughs, R.; Mohr, H. E.; Hubbard, J. D. *Phytophathology* **1979**, *69*, 1202.
Tancogne, J. *Ann. Tab.: Sect. 2* **1977**, *14*, 197.
Tanret, C. *C. R. Hebd. Seances Acad. Sci.* **1889**, *108*, 98.
Turner, W. B. "Fungal Metabolites"; Academic Press: New York, 1971; p 256.
U. S. Department of Agriculture 1975, Part 201, Federal Seed Act Regulations. Seed Testing Regulations. U.S. Department of Agriculture, Washington, DC.
U.S. Department of Agriculture, Federal Grain Inspection Service, Inspection Division Jan 1978, The Official United States Standards for Grains.
White, J. D.; Perkins, D. W.; Taylor, S. I. *Bioorg. Chem.* **1973**, *2*, 163.
Young, J. C. *Feedstuffs* **1979**, *51*, 23.
Young, J. C. *J. Environ. Sci. Health, Part B* **1981**, *B16*, 381.

Received for review February 28, 1983. Accepted May 23, 1983. Mention of a trademark of proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other suitable products.