Table I—Reagent Needed to Titrate Samples Subjected to Varying Pretreatment, ml.

A	B
0.23	0.24
0.24	0.25
0.28	0.30
0.25	0.29
0.23	0.22
0.28	0.24
0.25	0.23
0.27	0.24
0.24	0.30

authors found that a lyophilized B complex containing 10 mg. riboflavin could be titrated to an easily identified end point despite the color in the sample. Other types of sample have not been tested but it would seem that the method is applicable to any parenteral-type vial containing lyophilized or powdered sample low in water content, unless it is deeply colored or contains a substance which interferes with the titration, such as ascorbic acid.

Simple vials do not require the preliminary preparation described for the double-chambered vial, but can be titrated directly.

Table II—Determination of Water in Freeze-dried Acetylcholine Chloride–Mannitol Mixture

Blanks	Standards	Samples			
		Lot X		Lot Y	
Titer, ml.	Titer, ml.	Titer, ml.	H_2O, mg.	Titer, ml.	H_2O, mg.
0.07	0.45	0.22	0.77	0.23	0.83
0.07	0.44	0.28	1.09	0.18	0.56
0.08	0.45	0.21	0.72	0.17	0.50
0.07	0.45	0.38	1.63	0.19	0.61
0.09	0.45	0.22	0.77	0.25	0.93
Av. = 0.076	Av. = 0.448	0.20	0.66	0.17	0.50
		0.23	0.83	0.21	0.72

long time and might be expected to have absorbed some water not easily driven off.

(*b*) There is no significant change in K.F. titer even at 110° for 3 hr., either through volatilization of water through the center seal or through chemical reaction.

For simplicity, oven heating was adopted for the procedure.

Table II shows some results obtained by the proposed method, the values for water in samples of Lot X and Y being calculated using the average blank and standard titers.

A recalibrated pipet was used in preparing the water in methanol standard. A weighed quantity of standard hydrated sodium acetate might be considered as an alternate.

Applicability to Other Types of Sample—The

CONCLUSION

It is believed that the proposed titrimetric procedure is the method of choice for determining water in certain applicable cases.

REFERENCES

(1) Levy, G. B., Murtaugh, J. J., and Rosenblatt, M., *Ind. Eng. Chem., Anal. Ed.*, **17**, 193(1945).
(2) Wiberley, J. S., *Anal. Chem.*, **23**, 656(1951).
(3) Burger, H., and Polderman, J., *Pharm. Weekblad*, **100**, 1125(1965).
(4) Sobel, H., *Anal. Chem.*, **25**, 1756(1953).
(5) Alajos, V., *Gyogyszereszet*, **8**, 224(1964).

Keyphrases

Lyophilized substances—moisture in vials
Vials—moisture determination
Karl Fischer titration—analysis
Methanol—reagent

Preliminary Phytochemical Investigation of Wild Rice Ergot

By GREGORY T. SINNER* and LEE C. SCHRAMM†

Ergot parasitizing wild rice was analyzed for moisture, fat, and alkaloid content. Results indicated 4.6 percent moisture, 32 percent fat, and a total alkaloid content of approximately 0.1 mg. percent. The fungus was cultured in a modified *Claviceps* culture medium, but alkaloids could not be detected in culture filtrates.

Species of Claviceps have been found as parasites of nearly all members of the grass family (1).

Received September 25, 1967, from the College of Pharmacy, The University of Minnesota, Minneapolis, MN 55455
Accepted for publication December 19, 1967.
A portion of this paper was presented as the 1964 Kilmer Prize Paper to the Scientific Section, APhA, Academy of Pharmaceutical Sciences New York City meeting, August 1964.

* Present Address: Department of Chemistry, Dartmouth College, Hanover, NH 03755
† Present Address: School of Pharmacy, University of Georgia, Athens, GA 30601. To whom reprint requests should be directed.

The authors wish to acknowledge the assistance of Mrs. Doris Severn, Fergus Falls, Minn., and Mr. Harry Wilson, Spooner, Wis., for their collections of wild rice ergot.

The occurrence of this fungus on wild rice (*Zizania aquatica*) was first reported by Dennison in 1900 (2). In 1915, Fyles (3) published a preliminary morphological description of wild rice ergot and noted its host specificity. Although she was able to cause infestation of healthy wild rice by inoculation with conidial suspensions and honey dew from diseased wild rice, she was repeatedly unable to infect other healthy grains (normally susceptible to *Claviceps* infestations) with this organism. Presumably because of this, Fyles later referred to the ergot of wild rice as a distinct species, *Spermoedia zizaniae*, a

name which was never widely accepted. In 1959 Pantidou (4) published a complete description of wild rice ergot and proposed the presently accepted binomial, *Claviceps zizaniae* (Fyles) Pantidou, again as a species distinct from the common rye ergot *C. purpurea* (Fries) Tulasne. The consideration as a separate species results from discrete differences in physical characteristics of the sclerotia, and from the apparent host specificity of *C. zizaniae*.

Wild rice abounds in Minnesota and Wisconsin. Infestation of the natural stands of wild rice with *C. zizaniae* is common. The availability of sclerotia of wild rice ergot from local sources prompted the present phytochemical investigation for moisture, fat, and alkaloid content. An isolate of this fungus was grown in agitated submerged liquid culture, and the culture filtrate was examined for alkaloids.

EXPERIMENTAL

Sclerotia of wild rice ergot were collected from central Minnesota, near Fergus Falls, and from northern Wisconsin, near Spooner. These sclerotia corresponded to the description of Pantidou (4). The average size was 12 mm. in length (4–16 mm.) and 5 mm. in width, externally pink to black in color and having a brilliant purple fracture.

Moisture Determination—The sclerotia were ground in a Wiley Mill to a coarse powder. Moisture content was accomplished by determining weight loss after drying at 108° to constant weight. The average weight loss on drying was 4.6%.

Fat Determination—Samples of powdered wild rice ergot dried at 108° were extracted with petroleum ether by continuous extraction in a Soxhlet apparatus for 12 hr. Fat content was determined by loss of weight during extraction. The average fat content was 32% by weight.

Alkaloid Determination—The methods of Silber and Schulze (5) and Michelon and Kelleher (6) were used for the detection of alkaloids in wild rice ergot and on the filtrates of 7-day-old cultures of *C. zizaniae*.

Colorimetric analysis of the alkaloid content of wild rice ergot indicated the presence of alkaloids, but in a concentration of approximately 0.1 mg. %, the lower limit of detection of the analytical procedure. Water-soluble alkaloids appear to be absent in wild rice ergot, although they may have escaped detection because of a limited quantity of sample available for study. No indication of water-soluble alkaloids was observed chromatographically.

Investigation of Liquid Cultures—Individual sclerotia were sterilized by submersion in the following solutions for 30 sec. each: benzalkonium[1] chloride (1:500); sterile distilled water; 95% alcohol; 70% alcohol; 50% alcohol; sterile distilled water. After sterilization and rinsing, each sclerotium was peeled with a sterile scalpel; a small cubical portion (approximately 3 mm. on a side) was placed on a sterile agar slant of medium prepared according to Brady and Tyler (7). The slants were incubated at 26°; small portions of the mycelium were transferred to new medium monthly. Liquid cultures were prepared from 7-day-old slants by transferring a small portion of mycelium to 100 ml. of a sterile liquid medium modified slightly from Arcamone's "medium A" or preculture medium (8) consisting of 4% mannitol, 1% succinic acid, 0.1% neopeptone, 0.1% KH_2PO_4, 0.03% $MgSO_4$, and tap water, contained in a 500-ml. conical flask. The liquid cultures were incubated in a constant temperature room at 26° on a rotary shaker (300 r.p.m., 2.54-cm. diameter rotation). Filtrates of agitated submerged cultures of *C. zizaniae* were assumed not to contain ergot alkaloids since there was only a very weak reaction of the filtrate with the PDAB reagent. The color developed was not intense enough to be detectable in the analytical procedure.

Chromatographic Examination—Attempted identification of the alkaloids was accomplished on Silica Gel G thin-layer plates using two solvent systems comprised of benzene–ethanol (1:1) and (1:0.6) and on Alumina G thin-layer plates using solvents composed of chloroform, chloroform–*n*-butanol (10:1), and *n*-butanol. Each chromatoplate was examined under ultraviolet light to detect fluorescent substances, then sprayed with 0.2% para-dimethylaminobenzaldehyde in 18% HCl (PDAB) as a detection reagent.

On the silica gel plates, two alkaloid spots were observed. Although both yielded a blue fluorescence in ultraviolet light, they were readily differentiated on the basis of their reaction with the PDAB spray reagent. The alkaloid with the lower R_f value yielded a blue color with PDAB, while the other alkaloid gave a purple color with the reagent. The R_f values in the benzene–ethanol (1:1) system were 0.79 and 0.87; for the benzene–ethanol (1:0.6) system these values were 0.72 and 0.80.

On alumina plates, only one spot was observed. This spot also fluoresced blue and formed a purple or blue color with PDAB. The R_f value in the three solvent systems ranged from 0.93 to 0.96. Although other attempts were made to identify these alkaloids chromatographically, the results were inconclusive.

[1] Zephiran chloride, Winthrop Laboratories, New York, N. Y.

REFERENCES

(1) Brady, L. R., *Lloydia*, **25**, 1(1962).
(2) Dennison, R. H., *Pharm. Rev.*, **18**, 118(1900).
(3) Fyles, F., *Phytopathology*, **5**, 186(1915).
(4) Pantidou, M. E., *Can. J. Botany*, **37**, 1233(1959).
(5) Silber, A., and Schulze, T., *Pharmazie*, **8**, 675(1953).
(6) Michelon, L. E., and Kelleher, W. J., *Lloydia*, **26**, 192(1963).
(7) Brady, L. R., and Tyler, V. E., Jr., *ibid.*, **23**, 10(1960).
(8) Arcamone, F., Chain, E. B., Ferretti, A., Minghetti, A., Pannella, P., Tonolo, A., and Vero, L., *Proc Roy. Soc. (London), Ser. B.*, **155**, 26(1961).

Keyphrases

Ergot—wild rice fungus
Wild rice, *Zizania aquatica*—phytochemical properties
Cultures, liquid—*Claviceps zizaniae*
TLC—identity