$c = 12.264_3$ Å, and $\beta = 116.81_4°$. Intensities for 1725 (1558 observed) reflections with $2\theta < 50°$ were collected (General Electric XRD-5 diffractometer; MoKα radiation monochromatized by balanced zirconium and yttrium filters).

The iodine atoms were located from Patterson functions, and the complete molecule from three-dimensional Fourier synthesis. After block diagonal least-squares refinement of all observed data, the reliability index was 0.049.

The observed conformation of the *distal* triiodo-L-thyronine is shown in Fig. 1 along with that of the *proximal* form of triiodo-L-thyronine observed by Camerman and Camerman (*5*). Again the two phenyl rings are nearly perpendicular to each other, and the dihedral angles between the α ring and the C–O–C ether plane and the β ring and the C–O–C ether plane are 116° and 20°, respectively. The C–O–C angle is 120°. The structure is hydrogen bonded in the lattice by a network of hydrogen bonds through the carboxyl group and the amine of adjacent molecules. There are no short intermolecular distances involving the 3′ iodine in T_3 nor in the other two structures having the 3′ iodine in the *distal* position.

The observation of both the *distal* and *proximal* forms of T_3 in the solid state indicates that the two conformations are readily accessible in solution and that their relative energies are probably similar. This assumption has been further verified by Kollman (*10*) who, from molecular orbital calculations, found that the *proximal-distal* energy difference was on the order of 0.1 kcal/mole. The three crystalline examples of the *distal* conformation in various compositional derivatives of T_3 as opposed to the two *proximal* forms of T_3 crystallized from HCl points to the ease with which these two conformers can be selectively crystallized and that solvent effects may have some influence in stabilizing either form.

Because the *distal* conformation of T_3 has been shown to be stable, it is not necessary to propose that the rotation of the β ring is part of the T_3 receptor function (*5*). Furthermore, the readily accessible *distal* conformation does not throw into question the results of biological activity studies, nor does it require a modification for the manner of tissue uptake of T_3.

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References and Notes

1. E. C. Jorgensen, *Proc. Mayo Clinic* **39**, 560 (1964); N. Zenker and E. C. Jorgensen, *J. Amer. Chem. Soc.* **81**, 4643 (1959).
2. E. C. Jorgensen and P. E. Berteau, *J. Med. Chem.* **14**, 1199 (1972), and the previous papers in this series.
3. G. C. Schussler, *Science* **178**, 172 (1972).
4. L. B. Kier and J. R. Hoyland, *J. Med. Chem.* **13**, 1182 (1970).
5. N. Camerman and A. Camerman, *Science* **175**, 764 (1972).
6. V. Cody and W. L. Duax, *Acta Cryst.* **B28**, 2242 (1972).
7. ———, *Biochem. Biophys. Res. Commun.* **52**, 430 (1973).
8. V. Cody, unpublished results.
9. N. Camerman and A. Camerman, *Biochem. Biophys. Res. Commun.* **48**, 1433 (1972).
10. P. Kollman, private communication.
11. We thank M. Greiner for growing the crystals and Miss DeJarnette, Miss Strong, and Mrs. DeVine for data processing. We thank Dr. H. Hauptman for helpful discussions. Supported by NIH grant AM 15051 and a grant from Julia R. and Estelle L. Foundation, Inc., Buffalo, New York.

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12,13-Epoxy-Δ^9-Trichothecenes as the Probable Mycotoxins Responsible for Stachybotryotoxicosis

Abstract. Stachybotrys atra *cultures grown on oats produced five compounds toxic to brine shrimp; three are the sesquiterpenoid mycotoxins known as 12,13-epoxy-Δ^9-trichothecenes. One trichothecene is roridin E, a known metabolite of* Myrothecium verrucaria. *The other two were hydrolyzed to verrucarol, the product of roridin and verrucarin hydrolysis. Spectroscopic data indicate that the two remaining compounds are also 12,13-epoxy-Δ^9-trichothecenes. These metabolites are probably among those responsible for stachybotryotoxicosis.*

Stachybotryotoxicosis is a mycotoxicosis caused in animals by the ingestion of feed contaminated with certain toxin-producing strains of *Stachybotrys atra* (synonym *S. alternans*). The first reports of the disease came from the U.S.S.R. in 1931; the toxicosis attained enzootic proportions in some areas of the U.S.S.R. and was responsible for the deaths of thousands of horses. After an intensive search the cause of the toxicosis was attributed to certain metabolites of *S. atra*; these compounds have heretofore resisted chemical characterization (*1*).

More recently, Palyusik (*2*) has reported outbreaks of stachybotryotoxicosis among poultry, horses, and swine in Hungary. Forgacs and co-workers (*3*) have shown that the toxicosis can affect calves, sheep, swine, dogs, guinea pigs, rabbits, and mice.

Investigations of the mycotoxicosis have been reported and reviewed by Forgacs (*1*) and Palyusik (*2, 4*). Forgacs describes two forms of the toxicosis in horses: the typical, which is divided into three stages, and the atypical or shock form with one stage. In the typical form, the first stage involves stomatitis followed by fissures at the corner of the mouth, progressing to necroses. These lesions may subside before onset of the second stage, which is characterized by thrombocytopenia and increased blood clotting time. In some cases the blood fails to clot. In the third stage, thrombocytopenia and leukopenia increase, and failure of the blood to clot is observed in all cases. Body temperature increases, fresh necrotic areas appear, frequently with bacterial infection, and the animal usually dies within 1 to 6 days.

The atypical or shock form develops after ingestion of larger quantities of the toxin. Forgacs and co-workers (*3*) induced this form in one horse after it consumed 0.5 lb (1 kg = 2.2 lb) of straw infected with *S. atra* over a period of 10 days. Nervous disorders are the major visible symptoms of this form, which generally terminates in death.

Some of the symptoms produced in experimental animals as reported by Forgacs (*1*) and Palyusik (*2, 4*) are similar to those reported for the 12,13-epoxy-Δ^9-trichothecene group of toxic fungal metabolites; similar symptoms are dermal necrosis after topical application, leukopenia, thrombocytopenia, hemorrhages in the entire length of the digestive tract, and a noticeable lag period between the time of dosing and the appearance of visible symptoms. These symptoms varied with the test animals, quantities used in dosing, method of dosing, and observations made by the investigators. These observations led Bamburg and Strong (*5*) to postulate that the condition was caused by some members of the 12,13-epoxy-Δ^9-trichothecenes, a group of closely related sesquiterpenoids produced by various species of imperfect fungi.

According to Palyusik (*2*), the earliest work on the elucidation of the chemical structures of the mold metabolites was done by Fialkov and Serebryaniy in the Academy of Sciences, U.S.S.R. A compound given the trivial name stachybotryotoxin A was isolated as an amorphous powder from an ether extract of an *S. alternans* culture by precipitation with petroleum ether. This material was reported to have an empirical formula of $C_{25}H_{35}O_6$ (molecular weight, 430.3) or $C_{26}H_{38}O_6$ (molecular weight, 446.3). The presence of a steroidal ring system, an unsaturated lactone, and four hydroxyl groups was proposed. Palyusik further says that in 1960, Pashevich gave stachybotryotoxin A an empirical formula of $C_{24}H_{44}O_4$ with carboxylic acid functions present. Stachybotryotoxin B was described as crystallizing spontaneously upon evaporation of the ether extract, as being less soluble and less toxic than A, and as having three active hydrogens.

In our laboratory, samples prepared according to Fialkov and Serebryaniy's method for stachybotryotoxin A exhibited a multiplicity of spots when examined on silica gel thin-layer chromatographic plates developed with a mixture of isopropanol and chloroform (2 : 98, by volume).

Yuskiv (*6*) found that the isolate designated stachybotryotoxin A gave a color reaction with resorcinol. The reaction was used as a quantitative and qualitative test for the toxin. The sensitivity of the method was given as 5 μg/ml. However, we have found that spraying the developed thin-layer plates of the isolate designated stachybotyrotoxin A with the resorcinol reagent described by Yuskiv resulted in three red spots. These compounds were later separated from the toxic fraction by precipitation from a mixture of hexane and chloroform and were found to be nontoxic to the brine shrimp.

Seredyuk *et al.* (*7*) isolated a toxin from cultures of *S. alternans*. The toxin had a melting range of 62° to 69°C, a molecular weight of 302.8, and an empirical formula $C_{16}H_{27}O_5$. The material caused dermal inflammation in rabbits when applied to the ear skin, and decreased the germination rate of rye seeds. Seredyuk did not speculate on its chemical structure.

We have confirmed the postulate of Bamburg and Strong that some of the toxins produced by the fungus *S. atra* (*S. alternans*) are members of the 12,13-epoxy-Δ^9-trichothecene group. Five toxic compounds have been isolated from oat cultures inoculated with a *S. atra* strain (*8*) and grown at 20° to 22°C. After 4 weeks of growth, the cultures were air-dried and extracted with diethyl ether in a Soxhlet apparatus by the procedure for obtaining stachybotryotoxin A (*2*). The pale brown isolate was dissolved in chloroform and added to hexane (1 to 10 ratio) to give a nontoxic precipitate; all of the toxic compounds remained in solution. Silica gel column chromatography with a system of isopropanol and chloroform (1 : 99, by volume) followed by preparatory thin-layer chromatography resulted in the isolation of five toxic compounds. Each step in the isolation was monitored for toxicity by use of brine shrimp (*Artemia salina*) larvae (*9*). Toxicities to the brine shrimp larvae were shown to correspond with those of the rabbit skin and chick embryo bioassays (*10*).

A large quantity of the steroid ergosterol was isolated from stachybotryotoxin A prepared as reported above; this finding could explain the earlier report of the presence of a compound having a steroid nucleus. The well-known fungal metabolite mellein (*11*) was also isolated during the preparation of stachybotryotoxin A. The identity of mellein was confirmed by comparison with an authentic sample (*12*); it showed no toxicity toward the brine shrimp.

The toxic compounds isolated were designated satratoxins C, D, F, G, and H according to their descending order on silica gel thin-layer plates developed with a mixture of isopropanol and chloroform (2 : 98, by volume). The yields isolated from 2 kg of oats were 2 mg of C, 3 mg of D, 2 mg of F, 8 mg of G, and 12 mg of H. The amounts recovered do not represent the total of each toxin produced by the fungus, since each step in the purification resulted in some loss. Several other areas on the plates show significant toxicity in the brine shrimp and rabbit skin tests, but have not been processed to the point of yielding pure compounds. None of these compounds correspond to those isolated by Fialkov and Pashevich as mentioned in (*2*) or Seredyuk *et al.* (*7*). They did not give a color reaction with resorcinol. Three compounds from the nontoxic precipitate gave the positive resorcinol test on thin-layer plates.

The compound initially designated satratoxin D was identified as the known fungal metabolite roridin E by comparison with an authentic sample (*13*). Roridin E is a macrocyclic sesquiterpene produced by the soil fungus *Myrothecium verrucaria* (*14*). Both samples gave identical infrared, ultraviolet, nuclear magnetic resonance (NMR), and mass spectra. The thin-layer chromatographic characteristics of the two compounds were identical when several different solvent systems were used for development.

Satratoxins G and H were isolated in sufficient quantities for studies of chemical structure. Satratoxin G melted at 167° to 170°C and had an ultraviolet absorbance maximum at 256 nm ($\epsilon = 6{,}500$), a molecular weight 544.2064, and an empirical formula $C_{28}H_{32}O_{11}$ as determined by high resolution mass spectrometry. Satratoxin H had a melting range of 162° to 166°C, ultraviolet absorbance maxima at 225 nm ($\epsilon = 14{,}700$) and 255 nm ($\epsilon = 10{,}400$), a molecular weight 528.2375, and an empirical formula $C_{29}H_{36}O_9$, as determined by high resolution mass spectrometry. All ultraviolet absorbance measurements were made in methanol.

Upon hydrolysis with methanolic potassium hydroxide, both satratoxins G and H yielded verrucarol, the neutral hydrolysis product obtained when any of the roridins and verrucarins are hydrolyzed. The roridins and verrucarins are all macrocyclic esters of 4,15-dihydroxy-12,13-epoxy-Δ^9-trichothecene (verrucarol) and are compounds produced by the soil fungi *Myrothecium roridum* and *Myrothecium verrucaria* (*5*). Mass spectral and thin-layer chromatography comparisons with an authentic sample (*13*) confirmed that the major neutral hydrolysis product was verrucarol. The NMR spectra of both satratoxins G and H showed protons common to verrucarol, including the AB quartet in the region of δ 2.9 ($J_{AB} = 4.0$ hertz) attributed to the epoxide protons.

The initial attempts at isolating the acid products obtained on hydrolysis have not been completed. However, ultraviolet and NMR spectra demonstrated the presence of an α, β, γ, δ-diunsaturated ester group in both compounds. None of the roridins or verrucarins reported (*14*) correspond with satratoxins G and H.

Some of the toxic metabolites of *S. atra* are thus members of the 12,13-epoxy-Δ^9-trichothecene group and appear to be closely related to the macrocyclic roridins and verrucarins

produced by the *Myrothecium* species. We have evidence that satratoxins C and F are also members of the 12,13-epoxy-Δ^9-trichothecene series. Because so many members of the roridin-verrucarin series of sesquiterpenoid fungal metabolites are highly toxic and produce biological effects in experimental animals similar to those observed in stachybotryotoxicosis, it is reasonable to speculate that the members of this class of compounds which have been described in this report are, indeed, the chemical agents responsible for this disease.

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References and Notes

1. J. Forgacs, in *Microbial Toxins*, S. Kadis, A. Ciegler, S. J. Ajl, Eds. (Academic Press, New York, 1972), vol. 8, pp. 95–130.
2. M. Palyusik, *Acta Vet. Hung.* **20**, 57 (1970); Palyusik describes the work of V. Pashevich only as a thesis; and that of Fialkov as "Fungus disease of horses and people" (1949).
3. J. Forgacs, W. T. Carll, A. S. Herring, B. G. Mahlandt, *Trans. N.Y. Acad. Sci.* **20**, 787 (1958).
4. M. Palyusik and L. Bodon, *Acta Vet. Hung.* **20**, 289 (1970); M. Palyusik, *Sabouraudia* **8**, 4 (1970).
5. J. R. Bamburg and F. M. Strong, in *Microbial Toxins*, S. Kadis, A. Ciegler, S. J. Ajl, Eds. (Academic Press, New York, 1971), vol. 7, pp. 207–292.
6. R. V. Yuskiv, *Mikrobiol. Zh.* **28**, 68 (1966).
7. L. S. Seredyuk, A. A. Svishchuk, P. A. Moroz, *Fiziol. Biokhim. Osn. Vzaimodeistviya Rasten. Fitotosenozakh* **1971**, 133 (1971).
8. Strain No. M-1126, Food and Drug Administration, U.S. Department of Health, Education, and Welfare (HEW); also listed as Strain No. QM-1533, Quartermaster Corps, U.S. Army, and Strain No. 1006, Harvard University, Cambridge, Mass. We thank A. F. Schindler, Food and Drug Administration, HEW, for handling and growing the *S. atra* cultures. We also thank P. B. Mislivec, Food and Drug Administration, HEW, for confirming that the strain was *S. atra* before we began the study. The strain was examined for contamination at the start and conclusion of the study.
9. R. F. Brown, J. D. Wildman, R. M. Eppley, *J. Ass. Offic. Anal. Chem.* **51**, 905 (1968). The method as cited in this reference was used. Tests with ten 12,13-epoxy-Δ^9-trichothecenes and satratoxins gave a sensitivity range of 0.5 to 1.0 μg per milliliter of media for all of these compounds.
10. We thank C. W. Chung and M. J. Verrett, Division of Toxicology, Food and Drug Administration, HEW, for the toxicological studies of the *S. atra* extracts.
11. J. Blair and G. T. Newbold, *J. Chem. Soc.* (1955), p. 2871.
12. We thank U. L. Diener, Department of Botany and Microbiology, Auburn University Agricultural Experiment Station, Auburn, Alabama 36830, for the sample of mellein.
13. We thank C. Tamm, Institut für Organische Chemie der Universität Basel, Basel, Switzerland, for the samples of verrucarol and roridin E.
14. W. Zurcher and C. Tamm, *Helv. Chim. Acta* **49**, 2594 (1966); B. von Bohner, E. Fetz, E. Harri, H. P. Sigg, C. Stoll, C. Tamm, *ibid.* **48**, 1079 (1965); B. von Bohner and C. Tamm, *ibid.* **49**, 2527 (1966).
15. This paper is part of a dissertation to be submitted to the Graduate School, University of Maryland, by R. M. Eppley in partial fulfillment of the requirements for the degree of doctor of philosophy in chemistry.

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Prostaglandin Involvement in Hypothalamic Control of Gonadotropin and Prolactin Release

Abstract. *Prostaglandin E_2 (PGE_2) injected into the third ventricle of ovariectomized rats increased plasma luteinizing hormone dramatically and follicle stimulating hormone slightly. PGE_1 elevated prolactin; $PGF_{1\alpha}$ or $PGF_{2\alpha}$ had no effect. PGE_2 or PGE_1 injected directly into the anterior pituitary were ineffective. These results suggest that specific prostaglandins act at the hypothalamus to control pituitary hormone release.*

Prostaglandins (PG) are being implicated as intermediates in an ever-increasing number of physiologic systems, including several aspects of reproduction (*1*). Although participation of PG's in the regulation of ovarian function has been extensively studied, little is known about their role in relation to the hypothalamic pituitary axis. It was recently postulated that PG's may be involved in the process of ovulation because ovulation was blocked by inhibitors of PG synthesis (*2–4*). The site (or sites) of action of PG's in ovulation remains undetermined. Some data (*4, 5*) suggest that they have an effect directly on the ovaries. Other indirect evidence (*2, 3*) implies an effect on pituitary release of the gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH) which bring about ovulation.

Until now there has been no direct evidence for the involvement of PG's in the hypothalamic control of pituitary gonadotropin secretion. The hypothalamus regulates secretion of pituitary gonadotropins and prolactin by releasing specific neurohormones into the hypophyseal portal vessels. The neurohormones are then transported down the hypophyseal stalk via the portal vessels to the pituitary where they stimulate or inhibit release of particular anterior pituitary hormones (*6*). We now report that prostaglandins can affect pituitary secretion of LH, FSH, and prolactin and that this effect is probably mediated by the hypothalamus.

Female Sprague-Dawley rats (250 to 300 g at the time of treatment) were ovariectomized and housed in a room maintained at 24°C with a light-dark cycle of 14 hours of light and 10 hours of darkness. The rats were allowed free access to Purina rat chow and tap water. Two weeks after castration, each animal was implanted with a permanent cannula (23-gauge stainless steel), which was placed in the third ventricle or in each lobe of the anterior pituitary by means of a stereotaxic instrument (*7*). Proof of placement of the cannulas in the third ventricle was verified by leakage of cerebrospinal fluid upon removal of the mandril; intrapituitary implants were verified by observation of cannula tips within the pituitary tissue when the animal came to autopsy. Cannulas were considered properly placed when the tips extended into the tissue at least 0.5 mm from any lateral edge of the gland. Three to five days after surgery, 5 μg of PGE_1, PGE_2, or $PGF_{1\alpha}$ in a 5-μl volume [95 percent ethanol and 0.02 percent Na_2CO_3 (1:9)] were injected into the third ventricle. Five micrograms of $PGF_{2\alpha}$ (tromethamine salt) were injected in 5 μl of 0.9 percent NaCl. PGE_1 or PGE_2 (2.5 μg in 2.5 μl) were injected into each lobe of the anterior pituitary (5 μg in 5 μl total dose). Injections were made with a 10-μl Hamilton microsyringe connected by polyethylene tubing (PE 10) to a 30-gauge stainless steel cannula which fit snugly within and extended to the tip of the cannula. In all cases, fluid was injected at the rate of 2 μl/min. Heparinized blood samples (0.9 to 1 ml) were obtained by jugular puncture from lightly etherized animals before injection and 15, 30, and 60 minutes afterward. Concentrations of LH in the plasma were measured by radioimmunoassay by the method of Niswender *et al.* (*8*); concentrations of FSH and prolactin were measured by radioimmunoassay, with the kits provided by National Institute of Arthritis and Metabolic Diseases. The results were expressed in terms of the RP-1 rat pituitary reference preparations for FSH and prolactin and the NIH-LH-S1 standard for LH. Data were statistically analyzed by the paired *t*-test.

PGE_2 injected into the third ventricle