Engstrom, G. W., DeLance, J. V., Richard, J. L., Baetz, A. L., *J. Agric. Food Chem.* **23**, 244 (1975).
Forgacs, J., Kock, H., Carll, W. L., White-Stevens, R. H., *Am. J. Vet. Res.* **19**, 744 (1958).
Freeman, G. G., *J. Gen. Microbiol.* **12**, 213 (1955).
Garner, R. C., *J. Chromatogr.* **103**, 186 (1975).
Godtfredsen, W. O., Vangedal, S., *Acta Chem. Scand.* **19**, 1088 (1965).
Hayes, A. W., McCain, H. W., *Food Cosmet. Toxicol.* **13**, 221 (1975).
Hayes, A. W., Wilson, B. J., *Appl. Microbiol.* **16**, 1163 (1968).
Hood, R. D., Innes, J. E., Hayes, A. W., *Bull Environ. Contam. Toxicol.* **10**, 200 (1973).
Hsieh, D. P. H., Fitzell, D. L., Miller, J. L., Seiber, J. N., *J. Chromatogr.* 117, 474 (1976).
Kmieciak, S., *Z. Lebensm.-Unters Forsch.* **160**, 321 (1976).
Kovacs, F., Szathmary, C. S., Palyusik, M., *Acta Vet. Acad. Sci. Hung.* **25**, 223 (1975).
Krogh, P., in "Mycotoxins", Purchase, I. F. H., Ed., Elsevier, Amsterdam, 1974.
Madhavikutti, K., Shanmugasundaram, E. R. B., *Proc. Indian Acad. Sci., Sect. B* **68**, 261 (1968).
Mirocha, C. J., Christensen, C. M., in "Mycotoxins", Purchase, I. F. H., Ed., Elsevier, Amsterdam, 1974.
Moss, M. O., "Fungal Toxins", Vol. 6, Ciegler, A., Kadis, S., Ajl, S. J., Ed., Academic, New York, N.Y., 1971, p 381.
Moss, M. O., Hill, I. W., *Mycopath. Mycol. Appl.* **40**, 81 (1970).
Newberne, J. W., Bailey, W. S., Seibold, H. R., *J. Am. Vet. Med. Assoc.* **127**, 59 (1955).
Patterson, D. S. P., *Food Cosmet. Toxicol.* **11**, 287 (1973).
Pons, W. A., Jr., *J. Assoc. Off. Anal. Chem.* **59**, 101 (1976).
Purchase, I., Theron, J., *Food Cosmet. Toxicol.* **6**, 479 (1968).
Rao, G. H. R., Anders, M. W., *J. Chromatogr.* **84**, 402 (1973).
Richard, J. L., Thurston, J. R., Graham, C. K., *Am. J. Vet. Res.* **35**, 957 (1974).
Roberts, B. A., Patterson, D. S. P., *J. Assoc. Off. Anal. Chem.* **58**, 1178 (1975).
Romer, T. R., *J. Assoc. Off. Anal. Chem.* **56**, 1111 (1973).
Seiber, J. N., Hsieh, D. P. H., *J. Assoc. Off. Anal. Chem.* **56**, 827 (1973).
Seitz, L. M., *J. Chromatogr.* **104**, 81 (1975)
Smalley, E. B., Marasas, W. F. O., Strong, F. M., Bamburg, J. R., Nichols, R. E., Kosuri, N. R., *Proc. U.S.-J. Conf. Toxic Micro-Org., 1st* (1970).
Stack, M. E., Nesheim, S., Brown, N. L., Pohland, A. E., *J. Assoc. Off. Anal. Chem.* **59**, 966 (1976).
Stoloff, L., Nesheim, S., Yin, L., Rodricks, J. V., Stack, M., Campbell, A. D., *J. Assoc. Off. Anal. Chem.* **54**, 91 (1971).
Townsend, R. J., Moss, M. O., Peck, H. M., *J. Pharm. Pharmacol.* **18**, 471 (1966).
Ware, G. M., *J. Assoc. Off. Anal. Chem.* **58**, 754 (1975).
Ware, G. M., Thorpe, C. W., Pohland, A. E., *J. Assoc. Off. Anal. Chem.* **57**, 1111 (1974).
Wilson, D. M., "Mycotoxins and Other Fungal Related Food Problems", Rodricks, J. V., Ed., ACS Symposium *Adv. Chem. Ser.* **No. 149** 90–109 (1974).
Wilson, D. M., Tabor, W. H., Trucksess, M. W., *J. Assoc. Off. Anal. Chem.* **59**, 125 (1976).
Wogan, G. N., Edwards, G. S., Newberne, P. M., *Toxicol. Appl. Pharmacol.* **19**, 712 (1971).
Wogan, G. N., Newberne, P. M., *Cancer Res.* **27**, 2370 (1967).

Received for review November 22, 1976. Accepted March 2, 1977.

Synthesis of Radiolabeled T-2 Toxin

Edwin M. Wallace, Sadanand V. Pathre, Chester J. Mirocha,* Thomas S. Robison, and Stuart W. Fenton

Tritium-labeled T-2 toxin [4β,15-diacetoxy,8α-(3-methylbutyryloxy)-3α-hydroxy-12,13-epoxytrichothec-9-ene] was synthesized using a two-step process. First, the C-3 hydroxyl was oxidized to a ketone using either chromium trioxide–pyridine or dimethyl sulfide–*N*-chlorosuccinimide complex. In step two, the ketone was reduced with either tritiated sodium cyanoborohydride or tritiated sodium borohydride to a mixture of epimers which were separable by thin-layer chromatography. The α epimer (naturally occurring T-2 toxin) was formed predominantly, i.e., α to β ratio was 4 to 1. Final reduction with tritiated sodium borohydride yielded [3-^{3}H]T-2 toxin in 23% yield with a specific activity of 790 mCi/mmol. The labeled product, when equilibrated with 0.01 N HCl, did not lose the tritium, indicating that no labile tritium was incorporated into T-2 toxin. Further, the oxidation of [3-^{3}H]T-2 toxin resulted in total loss of activity in the corresponding ketone.

The metabolites of species of *Fusarium* are commonly found in foodstuff and are prominent because of their association with cases of mycotoxicoses of humans and animals. One such metabolite, T-2 toxin (1) [4β,15-diacetoxy,8α-(3-methylbutyryloxy)-3α-hydroxy-12,13-epoxytrichothec-9-ene] has been implicated as a mycotoxin responsible for the hemorrhagic syndrome and death of dairy cattle (Hsu et al., 1972). T-2 toxin causes an extreme dermal necrosis in most animals and is also one of the most potent inhibitors of protein synthesis in eucaryotic cells (Wei et al., 1974).

Our primary intesest was to prepare radiolabeled T-2 toxin with sufficient specific activity to enable us to study its metabolism in various animal species. We considered two basic methods of incorporating radioactive elements into T-2. One route was biosynthesis of 1 by feeding labeled precursors such as mevalonic acid (Jones and Lowe, 1960; Achilladelis and Hanson, 1972), farnasyl pyrophosphate (Evans et al., 1973), and trichodiene (Machida and Nozoe, 1972) to actively metabolizing cultures of *Fusarium*. Although the biosynthesis of radiolabeled T-2 toxin appears simple, it is time consuming, expensive, and results in a product with a relatively low specific activity.

Department of Plant Pathology and Department of Chemistry, University of Minnesota, St. Paul, Minnesota 55108.

Table I. Chemical Shifts[a] of Protons (ppm) in ^1H NMR Spectra of T-2 Toxin and Diacetoxyscirpenol and Their Dehydro Derivatives

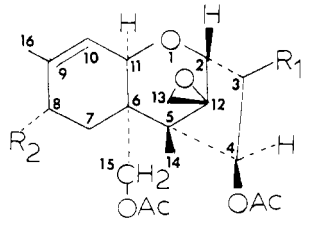
Compound	Positions			
	2	3	4	13
T-2 toxin[b]	3.48, D (5)	4.1 M	5.47, D (2.5)	2.70, 2.97, AB (4)
Diacetoxyscirpenol[c]	3.690 (5)	4.1	5.20, D (3)	2.78, 3.06, AB (4)
3-Dehydro-T-2 toxin	3.41, S		6.01, S	2.89, 3.07, AB (4)
3-Dehydrodiacetoxyscirpenol	3.54, S		6.00, S	2.97, 3.18, AB (4)

[a] Chemical shifts are relative to tetramethylsilane as an internal standard. Coupling constants are in the parentheses. [b] Data from Bamburg and Strong (1971). [c] Data from Sigg et al. (1965).

This prompted us to examine an alternative method, namely, oxidation of the secondary hydroxyl of **1** to a ketone **3** which could then be reduced to an epimeric mixture of **1** and **2** by a tritiated metal hydride. The latter transformations are often simple and result in high yields and specific activity.

RESULTS AND DISCUSSION

Oxidation of secondary alcohols of 12,13-epoxytrichothecenes by Jones reagent (Bowers et al., 1953) in acetone has been reported earlier (Sigg et al., 1965; Bamburg and Strong, 1971); however, this reagent was not satisfactory in our tests because it caused extensive rearrangement and hydrolysis of the T-2 toxin. The treatment of **1** with a



	R_1	R_2
1	H, OH	$-OCOCH_2CH(CH_3)_2$
2	OH, H	$-OCOCH_2CH(CH_3)_2$
3	=O	$-OCOCH_2CH(CH_3)_2$
4	H, OH	H
5	=O	H
6	T, OH	$-OCOCH_2CH(CH_3)_2$

T = ^3H

chromium trioxide–pyridine complex (Poos et al., 1953) in methylene chloride gave the desired ketone **3** in 80% yield; however, the reaction was rather sluggish, requiring 72 h for completion at room temperature and isolation of the product was tedious. Oxidation with pyridinium chlorochromate ($C_5H_5NCrO_3Cl$) as reported by Corey and Suggs (1975) was attempted. The reaction was carried out in methylene chloride in the presence of sodium acetate for 30 min and produced **3** in 50% yield as determined by GLC; a longer reaction time did not improve the yield. The reaction in the absence of sodium acetate was too sluggish to be synthetically useful. Finally, the complex of dimethyl sulfide–*N*-chlorosuccinimide (Corey and Kim, 1972) was found to be a suitable oxidant for T-2 toxin. The complex oxidized **1** to **3** (3-dehydro-T-2 toxin) in 94% yield in 2 h.

3-Dehydro-T-2 Toxin. The mass spectrum of the isolated product (presumably 3-dehydro-T-2 toxin) showed a weak molecular ion at m/e^+ 464, a significant loss of acetoxyl, acetic acid, and isovaleric acid similar to that of T-2 toxin. The product yielded *O*-methyloxime when reacted with methoxyamine hydrochloride, the mass spectrum of which displayed a strong molecular ion at m/e^+ 493 and significant fragments at m/e^+ 462, 433, and 391 due to loss of OCH_3, CH_3COOH, and $(CH_3)_2CHCH_2COOH$, respectively. The comparison of ^1H NMR spectra (Table I) of **3** with that of **1** indicated that the doublets due to C-2 and C-4 protons at 3.48 and 5.47 ppm of **1** had collapsed to singlets at 3.41 and 6.01 ppm, respectively, in the oxidized product. Also, the signals due to C-3 proton of **1** disappeared upon oxidation to **3**. The other ^1H NMR spectral features in **1** and **3** were almost identical, indicating **3** is 3-dehydro-T-2 toxin. Similar observations were noted when diacetoxyscirpenol **4** was oxidized to 3-dehydrodiacetoxyscirpenol **5** (Sigg et al., 1965).

The ketone **3** also reacted quantitatively with *N,O*-bis(trimethylsilyl)acetamide, a trimethylsilyl (Me_3Si) donor, and with *N*-methylbis(trifluoroacetamide) to form Me_3Si–enol ether and enol trifluoroacetate, respectively. These observations coupled with the fact that the isolated ketone **3** exhibited an extensive tailing effect on TLC indicated that the ketone **3** is highly enolizable; however, we were unable to observe the enolized form of **3** in $CDCl_3$ by ^1H NMR.

Reduction with $NaBH_3CN$. Preliminary results indicated that the ketone **3** can be reduced in fair yields (60%) to the epimeric mixture of T-2 toxin (**1** and **2**). The gas chromatographic analysis of the mixture indicated that the α epimer (identical with naturally occurring T-2 toxin) was formed predominantly and that the epimeric distribution of α to β was 4 to 1. The mixture was then resolved on TLC. Reduction of the ketone **3** with NaB^3H_3CN (sp act. 22 μCi/mM) gave the desired epimer **1** with a specific activity of 21.3 μCi/mM.

Reduction with NaB^3H_4. Attempts to prepare NaB^3H_3CN with a higher specific activity (~10 Ci/mM) according to the procedure of Borch et al. (1971) were unsuccessful. Therefore, the reduction with sodium borohydride was evaluated in the hope that NaB^3H_4 (sp act. 10 Ci/mM) available from the commercial source would yield the labeled toxin **6** in sufficiently higher yields and greater specific activity. The reduction of **3** occurred in fair yields, but it was noted that the rate of hydrolysis of $NaBH_4$ in methanol was too high and tended to decrease the net incorporation of tritium into the molecule **1**. Reduction under alkaline conditions was avoided since the starting material **3** and the product **1** are easily hydrolyzed in base. The use of ethanol did not increase the specific activity of the T-2 toxin but reduction in isopropanol seemed a suitable alternative. The reaction in isopropanol at 38 °C yielded the desired epimer in 23% yield with a sp act. of 790 mCi/mmol.

EXPERIMENTAL SECTION

Oxidation of T-2 Toxin to 3-Dehydro-T-2 Toxin. *Dipyridine–Chromium(VI) Complex.* A solution of T-2 toxin (3.1 mg) in 1 mL of dry methylene chloride was added, with stirring, to a 5% suspension of dipyridine–

chromium(VI) complex in 2 mL of dry methylene chloride at room temperature. The mixture was stirred for 66 h, diluted with 5 mL of methylene chloride, and washed with water, followed by 0.1 N hydrochloric acid. The methylene chloride layer was dried over anhydrous sodium sulfate. Gas chromatographic analysis of this layer indicated a single product. Evaporation of the solvent yielded 2.5 mg (80%) of the ketone 3. Calcd for $C_{24}H_{32}O_9$; mass spectrum, m/e^+ 464.

Dimethyl Sulfide–N-Chlorosuccinimide. Dimethyl sulfide (0.14 mL, 1.4 mmol) was added to a stirred suspension of 133 mg (1.0 mmol) of *N*-chlorosuccinimide in 10 mL of dry toluene at 0 °C under dry nitrogen. A white precipitate appeared immediately after the addition of dimethyl sulfide. The mixture was cooled to −25 °C (CCl_4–dry ice) and a solution of 300 mg (0.64 mmol) of T-2 toxin in 8 mL of toluene was added dropwise. The stirring was continued for 2 h and then a solution of 101 mg (1.4 mmol) of Et_3N in 0.5 mL of toluene was added dropwise. The cooling bath was removed and after 5 min, 20 mL of diethyl ether was added. The organic layer was washed with 10 mL of 0.1 N HCl and twice with 15 mL of water. Evaporation of the dried organic layer yielded 285 mg of gum (96% yield). Analysis by GLC and mass spectra and NMR indicated the product was 3-dehydro-T-2 toxin. Attempts to crystallize the ketone were unsuccessful. The compound deteriorated slowly during storage.

O-Methyloxime. The methyloxime of **3** was prepared as described by Mirocha et al. (1974) for zearalenone. The GLC analysis indicated almost quantitative conversion to the oxime. Calcd for $C_{25}H_{35}O_9N_1$; mass spectrum, m/e^+ 493.

Reduction of 3-Dehydro-T-2 Toxin. *A.* NaB^3H_3CN. The tritiated sodium cyanoborohydride was prepared from ^3H_2O as described by Borch et al. (1971). The sp act. was 0.35 μCi/mg. Reduction of 15.3 mg (33.6 mol) of **3** with 2.17 mg (34.4 μmol) of NaB^3H_3CN gave tritiated T-2 toxin **6** (α epimer) in 11% yield with a sp act. of 21 μCi/mmol (0.046 μCi/mg). The product had a mass spectrum and R_f value by TLC identical with those of an authentic sample of T-2 toxin.

B. NaB^3H_4. Tritiated sodium borohydride (14 μmol, 100 mCi) was weighed into a one dram vial containing a stirring bar and 27 mg (58 μmol) of the ketone **3** in 1.2 mL of isopropanol. The reaction mixture was diluted with 4 mL of chloroform and washed with 3 mL of 0.3 N HCl. The aqueous layer was extracted with 3 × 4 mL of chloroform, and the chloroform layers were dried and evaporated to dryness. The naturally occurring α epimer was separated from the β epimer by successive thin-layer chromatography in 95:5 $CHCl_3$–ethanol. Nonradioactive T-2 toxin (13.1 mg) was added after the initial chromatographic step to aid in complete recovery of the tritiated T-2 toxin. The 3-[^{3}H]T-2 toxin was obtained in 23% yield with a specific activity of 790 mCi/mmol.

LITERATURE CITED

Achilladelis, B., Hanson, J. R., Jr., *J. Chem. Soc., Perkin Trans. 1*, 1425 (1972).
Bamburg, J. R., Strong, F. M., in "*Microbial Toxins*", Vol. VII, Kadis, S., Ciegler, A., Ajl, S. J., Ed., Academic Press, New York, N.Y., 1971.
Borch, R. F., Bernstein, M. D., Durst, H. D., *J. Am. Chem. Soc.* **93**, 2897 (1971).
Bowers, A., Halsall, T. G., Jones, E. R. H., Lemin, A. J., *J. Chem. Soc.*, 2548 (1953).
Corey, E. J., Kim, C. U., *J. Am. Chem Soc.* **94**, 7586 (1972).
Corey, E. J., Suggs, J. W., *Tetrahedron Lett.* **31**, 2647 (1975).
Evans, R., Holtom, A. M., Hanson, J. R., *J. Chem. Soc., Chem. Commun.*, 465 (1973).
Hsu, I.-C., Smalley, E. B., Strong, F. M., Ribelin, W. E., *Appl. Microbiol.* **24**, 684 (1972).
Jones, E. R. H., Lowe, G., *J. Chem. Soc.*, 2959 (1960).
Machida, Y., Nozoe, S., *Tetrahedron* **28**, 5113 (1972).
Mirocha, C. J., Schauerhamer, B., Pathre, S. V., *J. Assoc. Off. Anal. Chem.* **57**, 1104 (1974).
Poos, G. I., Arth, G. E., Beyler, R. E., Sarett, L. H., *J. Am. Chem. Soc.* **75**, 422 (1953).
Sigg, H. P., Mauh, R., Flury, E., Hauser, D., *Helv. Chim. Acta* **48**, 962 (1965).
Wei, C. M., Hansen, B. S., Vaughan, M. H., Jr., McLaughlin, C. S., *Proc. Natl. Acad. Sci. U.S.A.* **71**, 713 (1974).

Received for review January 10, 1977. Accepted March 17, 1977. Scientific Journal Series Paper No. 9840, Minnesota Agricultural Experiment Station. This research was supported by U. S. Public Health Service Contract FDA 223-74-7229. Part of this paper was presented at the 170th National Meeting of the American Chemical Society Chicago, Ill. Aug 24–29, 1975.

Metabolites of *Alternaria alternata*: Ergosterol and Ergosta-4,6,8(14),22-tetraen-3-one

Larry M. Seitz* and Joseph V. Paukstelis

Ergosterol and ergosta-4,6,8(14),22-tetraen-3-one (ETO) were identified as metabolites of *Alternaria alternata* isolates from sorghum grain and wheat. Ergosterol contents ranged from 40 to 330 μg/g and ETO from 1.8 to 6.6 μg/g in 21-day cultures from 4 isolates of *A. alternata* as determined by high pressure liquid chromatography. The compounds were identified by thin-layer chromatography, and by ultraviolet, infrared, mass, and nuclear magnetic resonance (proton and carbon-13) spectroscopy. Carbon-13 magnetic resonance spectra of these metabolites are presented.

Although ergosterol is considered to be a metabolite common to all fungi (Weete, 1974), its production by *Alternaria brassicicola*, *A. kikuchiana*, and *A. alternata* (Fries) Keissler (formerly *A. tenuis* Auct.) was reported only recently (Aizina et al., 1974; Starratt, 1976). We add further evidence for production of ergosterol by *Alternaria*, specifically *A. alternata* isolated from sorghum grain and wheat.

We also report the first evidence for production of ergosta-4,6,8(14),22-tetraen-3-one (ETO) by *Alternaria*

U.S. Grain Marketing Research Center, Manhattan, Kansas 66502 (L.M.S.) and Department of Chemistry, Kansas State University, Manhattan, Kansas 66506 (J.V.P.).