attributable to the fragmentations *a* and *b*, and a strong peak at *m/e* 290, corresponding to the fragment ion **3**. The position of the hydroxymethacrylate group was assigned to C-8 on the basis of the PMR spectum of **1** [δ 3.86 (1H, *t*, J = 10 Hz, H-6) and δ 5.24 (1H, *m*, H-8)].

The physical data of **1** were similar to those of vernodalin (**4**), previously isolated from *V. amygdalina* [1]. The presence of a methoxycarbonyl group in **1** suggested that the δ-lactone of **4** was opened and esterified by methanol.

Hydrolysis of **1** in acidic methanol yielded **5**, previously obtained by reaction of vernolepin (**6**) with methanol [2,3]. Therefore, the structure of vernodalol is represented by **1**.

EXPERIMENTAL

Mp's were uncorr. IR spectra were measured as KBr pellets and the UV spectra were determined in EtOH soln. The PMR spectra were taken in $CDCl_3$ solns. Chemical shifts were reported in δ-value using TMS as an internal reference.

Isolation of vernodalol. The dried seeds of *Vernonia anthelmintica* Willd (500 g) were extracted with Et_2O and the combined extracts were concd under red. press. to a thick oil (30 g). The crude material was chromatographed repeatedly on Si gel. Elution with $CHCl_3$–MeOH (98:2) gave **1** as colourless needles (2.8 g); mp 133–134° (from $CHCl_3$); $[\alpha]_D$ +36.5° (c = 1.00, $CHCl_3$); λ_{max}^{EtOH} 210 nm (ε 16500); ν_{max}^{KBr} cm^{-1}: 3540, 1730, 1710, 1690, 1620 and 1160; MS *m/e*: 392 (M^+), ^{13}C-NMR; 144.6 (C-1), 117.7 (C-2), 54.0 (C-5), 71.5 (C-6), 57.0 (C-7), 72.6 (C-8), 40.7 (C-9), 42.5 (C-10), 73.5 (C-14), 63.1 (—CH_2OH) and 54.1 (—COO$\underline{C}H_3$); C=O at 169.6, 167.4 and 166.6; CO—$\underline{C}$=CH_2 at 135.3, 141.5 and 144.4; C=$\underline{C}H_2$ at 126.4, 130.7 and 136.4 ppm; (found; C, 61.12, H, 6.15, $C_{20}H_{24}O_8$ requires C, 61.21, H, 6.17%).

Acetylation of vernodalol. **1** (115 mg) was acetylated in the usual manner. The crude product was recrystallized from EtOH to give colourless needles of **2** (32 mg); mp 143–144°; ν_{max}^{KBr} cm^{-1}; 1740, 1720, 1710, 1620, 1220, 800 and 680 PMR: δ 1.90 (3H, *s*, -OAc) 2.04 (3H, *s*, —OAc), 3.72 (3H, *s*, —COOMe), 5.46 (1H, *t*, J = 10 Hz, H-6) and 5.52 (1H, *m*, H-8).

Hydrolysis of vernodalol. A soln of **1** (65 mg) in MeOH (4 ml) containing conc HCl (1 ml) was refluxed for 22 hr. Removal of the solvent afforded a crystalline residue which was recrystallized from MeOH–Et_2O to give colourless needles of **5** (27 mg); mp 174°. This material was identical with **5** by mmp and TLC comparison.

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REFERENCES

1. Kupchan, S. M., Hemingway, R. J., Karim, A. and Werner, D. (1969) *J. Org. Chem.* **34**, 3908.
2. Kupchan, S. M., Hemingway, R. J., Karim, A. and Werner, D. (1968) *J. Am. Chem. Soc.* **90**, 3596.
3. Kupchan, S. M., Hemingway, R. J., Karim, A. and Werner, D. (1969) *J. Org. Chem.* **34**, 3903.

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A NEW MYCOTOXIN FROM *FUSARIUM*

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Key Word Index—*Fusarium tricinctum*; mould; mycotoxin; 12,13-epoxytrichotec-9-ene-derivative.

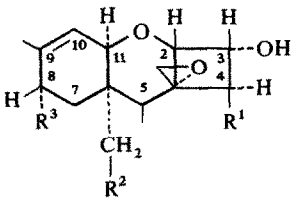
Abstract—A new mycotoxin was isolated from a strain of *Fusarium tricinctum* cultivated for four months on a grain mixture. It was shown to be 4β, 8α-diacetoxy-12,13-epoxytrichotec-9-ene-3α, 15-diol by PMR and MS-analysis.

In connection with work on the detection and analysis of *Fusarium* mycotoxins the toxin producing properties of several *Fusarium tricinctum* strains originating from different parts of Finland were examined. From one such strain the known mycotoxin T-2 (**1**) was isolated [1]. When the incubation time was extended to four months two other known toxins, HT-2 (**2**) and neosolaniol (**3**), as well as a new toxin were obtained; the structure determination of the latter forms the subject of the present report. The compound could not be crystallized although TLC indicated a high degree of purity. Insufficient was obtained for the preparation of derivatives, with the exception of the bis-TMS ether for GC–MS analysis. The PMR spectrum was very similar to the corresponding spectra of **1** [2], **2** [3] and **3** [4]. There was also a great similarity between the IR spectrum of the new toxin and those of **1** [5], **3** [4] and diacetoxyscirpenol (**4**) [6]. The compound was clearly a derivative of 12,13-epoxytrichothec-9-ene.

The MS showed a weak parent peak at *m/e* 382, corresponding to $C_{19}H_{26}O_8$, the main fragment ions occurring at *m/e* 322 [M^+-AcOH], 292 [322-CH_2O] and 232 [292-AcOH].

This fragmentation pointed to the presence of two acetoxyl groups. which was also supported by the occurrence of two three proton singlets in the PMR spectrum at δ2.09 and 2.16. From the information given above it can be concluded that the toxin was the diacetate of a 12,13-epoxytrichothec-9-enetetraol, and hence is isomeric with neosolaniol [4].

The signals in the region δ5–6 in the PMR spectrum were almost identical to the corresponding signals in the spectrum of **1** [2], [5], regarding both the chemical shifts and the splitting patterns. The acetoxyl groups were consequently located at 4β and 8α. A doublet of doublets at δ4.24 corresponded closely to the signal assigned to βH-3 in **1** [2], **2** [3] and **4** [6, 7], indicating that one of the hydroxyl groups was 3α. The second hydroxyl group was placed at C-15 because of a two proton AB-quartet centered at δ3.75 with J = 12.8 Hz.



	R^1	R^2	R^3
1	OAc	OAc	$OOCCH_2CHMe_2$
2	OH	OAc	$OOCCH_2CHMe_2$
3	OAc	OAc	OH
4	OAc	OAc	H
5	OAc	OH	OAc

Similar signals were reported for verrucarol [8] and for T-2-tetraol [2], both having C-15 as a primary hydroxyl function. The signals for the remaining protons were also in close agreement with the expected values [9].

That C-15 carried a free hydroxyl group gained further support from the MS which showed a fragment ion formed by loss of CH_2O, as indicated above. In the MS of the bistrimethylsilyl ether there was a peak at *m/e* 272, which was attributed to the fragmentation [M^+-AcOH–HOTMS–CH_2OTMS].

It can thus be concluded that the compound was 4β, 8α-diacetoxy-12,13-epoxytrichothec-9-ene-3α, 15-diol (**5**) or its enantiomer. Taking into account that all trichothecene derivatives hitherto isolated from natural sources have been found to have the same absolute configuration [9], it is assumed that this also holds for the present compound, the structure of which is therefore as depicted in **5**.

EXPERIMENTAL

Isolation of 4β, 8α-diacetoxy-12,13-epoxytrichothec-9-ene-3α, 15-diol (**5**). The toxin producing strain, *Fusarium tricinctum* (Corda) Saccardo (strain 72187, Dept. Plant Pathology), was grown for a period of 4 months according to [10]. Dry grain mixture (1 kg) was homogenized in EtOAc at room temp. (Waring blender). The red EtOAc filtrate was evapd and washed as in [1]. Fractionation of toxins was on a Kieselgel 60 (Merck), size C prepacked column, eluting with EtOAc. Every second tube was monitored using U-cells (human amnion cells cultured as described [10]) and toxic fractions were collected. Purification of the new toxin was on similar columns first using 4% EtOH in $CHCl_3$ as eluent and in a second column Me_2CO–hexane (1:1). The purity of the material was checked by TLC (Si gel); R_f 0.4 in EtOAc–petrol (3:1) showing skyblue fluorescence in the UV (254 nm) after spraying with *p*-anisaldehyde and heating [11]. A similar colour reaction is given by T-2 toxin. IR ν_{max}^{Film} cm^{-1}: 3450 (*s*), 2940 (*s*), 1720 (*s*), 1230 (*s*). PMR (100 MHz; $CDCl_3$): δ0.84 (3H, *s*; 14-Me), 1.75 (3H, *br s*; 16-Me), 1.96 (1H, *d*, J = 15.4 Hz; αH-7), 2.09 (3H, *s*), 2.16 (3H, *s*), 2.34 (1H, *dd*, J = 15.4 and 5.8 Hz; βH-7), 2.80 (1H, *d*, J = 3.9 Hz; H-13), 3.04 (1H, *d*, J = 3.9 Hz; H-13), 3.60 (1H, *d*, J = 12.8 Hz; H-15), 3.66 (1H, *d*, J = 4.8 Hz; H-2), 3.91 (1H, *d*, J = 12.8 Hz; H-15), 4.24 (1H, *dd*, J = 4.8 and 3.2 Hz; βH-3), 4.27 (1H, *d*, J = 5.7 Hz; H-11), 5.36 (1H *br d*, J = 5.8 Hz; βH-8), 5.4 (1H, *d*, J = 3.2 Hz; αH-4), 5.82 (1H, *br d*, J = 5.7 Hz; H-10). MS (probe) 75 eV, *m/e* (rel. int.): 382 (M^+; 1.5), 322 (17), 292 (19), 232 (100).

Bistrimethylsilyl ether. Prepared from BSA. MS (GC–MS) 22.5 eV, *m/e* (rel. int.): 466 (M^+-60; 60), 376 (56), 272 (83), 185 (100).

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REFERENCES

1. Niku-Paavola, M.-L., Ilus, T., Ward, P. J. and Nummi, M. (1977) *Ann. Nutr. Aliment.* in press.
2. Bamburg, J. R., Riggs, N. V. and Strong, F. M. (1968) *Tetrahedron* **24**, 3329.
3. Bamburg, J. R. and Strong, F. M. *Phytochemistry* (1969) **8**, 2405.
4. Ishii, K., Sakai, K., Ueno, Y., Tsunoda, H. and Enomoto, M. (1971) *Appl. Microbiol.* **22**, 718.
5. Ueno, Y., Ishii, K., Sakai, K., Kanaeda, S., Tsunoda, H., Tanaka, T. and Enomoto, M. (1972) *Japan. J. Exp. Med.* **42**, 187.
6. Sigg, H. P., Mauli, R., Flury, E. and Hauser, D. (1965) *Helv. Chim. Acta* **48**, 962.
7. Tidd, B. K. (1967) *J. Chem. Soc. C* 218.
8. Gutzwiller, J., Mauli, R., Sigg, H. P. and Tamm, Ch. (1964) *Helv. Chim. Acta* **47**, 2234.
9. Bamburg, J. R. and Strong, F. M. (1971) in *Microbial Toxins* (Kadis, S., Ciegler. A. and Ajl, S. J. eds) Vol. 7, p. 207. Academic Press, New York.
10. Korpinen, E.-L. and Uoti, J. (1974) *Acta Pathol. Microbiol. Scand. Sect. B* **82**, 1.
11. Scott, P. W., Laurence, J. W. and von Walbeck, W. (1970) *Appl. Microbiol.* **20**, 839.