Phytochemistry, 1977, Vol 16, pp 1838–1839. Pergamon Press. Printed in England.

STUDIES ON A BITTER PRINCIPLE FROM *VERNONIA ANTHELMINTICA*

Y. ASAKA*, T. KUBOTA* and A. B. KULKARNI†

*Department of Chemistry, School of Medicine, Kinki University, Sayama-cho, Minami-Kawachi-gun, Osaka, Japan, 589;
†Department of Chemistry, University of Bombay, Vidyanagari, L, kmanya Gangadhar, Tilak, Bhavan, Bombay, 400029, India

(*Revised received* 20 *May* 1977)

Key Word Index—*Vernonia anthelmintica*; Compositae; bitter elemanolide lactone; vernodalol.

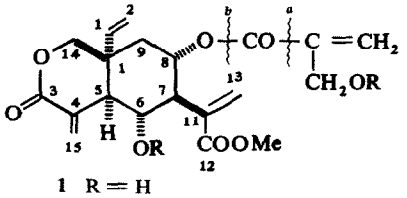
Repeated chromatography of ether extracts of the dried seeds of *V. anthelmintica* (Indian name, Kali Jiri) on silica gel gave crystalline vernodalol, a new elemanolide lactone, which is assigned the structure **1**.

Vernodalol (**1**), $C_{20}H_{24}O_8$ (M^+ 392), crystallized from chloroform, mp 133–134°, $[\alpha]_D$ +36.5° (c = 1.00, $CHCl_3$). It contained an α,β-unsaturated δ-lactone moiety [λ_{max}^{EtOH} 210 nm (ε 16500), ν_{max} 1730 cm^{-1}] and a methoxycarbonyl group [ν_{max} 1710 cm^{-1}, $\delta(CDCl_3)$ 3.72 (3H, *s*)]. The IR spectrum also displayed characteristic bands at 3540 (hydroxyl group), 1690 (terminal methylene group) and 1620 cm^{-1} (vinyl group). The PMR signals are summarized in Table 1 and were assigned by decoupling experiments.

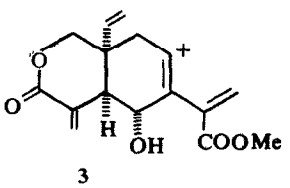
Compound **1** was acetylated by the usual manner to afford a crystalline diacetate **2** (mp 143–144°). Its PMR spectrum was similar to that of **1**, but had two acetate methyl signals at δ 1.90 and 2.04 and the signals of H-6 and H-8 were at δ 5.46 (*t*, J = 10 Hz) and at δ 5.52 (*m*) respectively. Since it showed no D_2O-exchangeable signals, **1** has two hydroxyl groups. The signals at δ 4.18, 5.80 and 6.10 in **1** suggested the presence of a hydroxymethacrylate group. This was supported by the MS, which showed prominent peaks at *m/e* 57 and 85,

Table 1. PMR data (γ, ppm) of vernodalol (**1**)

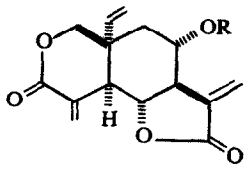
Chemical shift		Coupling constant	Assignment
1.56	1H	*dd* J = 12 and 14 Hz	H-9α
2.00	1H	*dd* J = 5 and 14 Hz	H-9β
2.50	1H	*dd* J = 2 and 10 Hz	H-5
2.70	1H	*dd* J = 10 and 12 Hz	H-7
3.86	1H	*t* J = 10 Hz	H-6
4.30	1H	*d* J = 13 Hz	H-14α
4.71	1H	*d* J = 13 Hz	H-14β
5.12	3H	*m*	H-1, H-2a and H-2b
5.24	1H	*m*	H-8
5.68	1H	*br.s*	H-13a
5.72	1H	*d* J = 2 Hz	H-15a[1]
6.24	1H	*s*	H-15b[1]
6.52	1H	*d* J = 2 Hz	H-13b
2.65	2H	*s* exchangeable with D_2O	OH
3.72	3H	*s*	COOMe
4.18	2H	*br.s*	CH_2OH
5.80	1H	*m*	$CO-C(=CH_2)-CH_2OH$
6.10	1H	*m*	



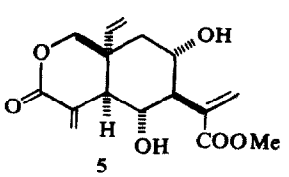
1 R = H
2 R = Ac



3



4 $R = COC(=CH_2)CH_2OH$



5

6 R = H

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.

Acknowledgements—The authors are grateful to the late Professor S. M. Kupchan (Virginia University) for an authentic sample of compound **5** and IR and PMR charts of vernodalin. We also thank Dr. T. Kamikawa for PMR measurements and Dr. I. Kubo for ^{13}C-NMR measurements.

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.

Phytochemistry, 1977, Vol. 16, pp. 1839–1840. Pergamon Press. Printed in England.

A NEW MYCOTOXIN FROM *FUSARIUM*

TAINA ILUS*, PHILIP J. WARD*, MARTTI NUMMI*, HERMAN ADLERCREUTZ† and JARL GRIPENBERG‡

* Biotechnical Laboratory, Technical Research Centre of Finland, Box 192, SF-00121 Helsinki 12, Finland; † Department of Clinical Chemistry, University of Helsinki, SF-00290 Helsinki 29, Finland; ‡ Department of Chemistry, Helsinki University of Technology, SF-02150 Espoo 15, Finland

(*Received* 24 *May* 1977)

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].