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NEW TRICHOTHECENE MYCOTOXINS OF Fusarium sporotrichioides (MC-72083)

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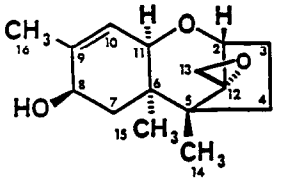
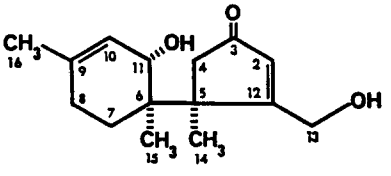
Abstract: Isolation and characterization of three new trichothecene mycotoxins from Fusarium sporotrichioides MC-72083 are reported. Structure and n.m.r. assignments were made using COSY, NOESY and INAPT as well as other spectroscopic techniques.

The trichothecenes are a growing class of toxic sesquiterpenoids produced by a variety of fungi, including Fusarium, Stachybotrys, Trichothecium, Myrothecium and Cephalosporium[1]. They are responsible for alimentary toxic aleukia, vomiting, skin inflammation, weight loss and death in humans and agricultural animals.[2] Detailed studies[3,4] suggest that the toxicoses induced in animals by ingestation of Fusarium infected wheat are not related to the amount of the commonly occurring Fusarium mycotoxins (i.e. deoxynivalenol, zearalenone, etc.) present. This indicates that the toxicity may arise from new minor secondary metabolites and/or synergistic effects.[5] Recent efforts in our laboratories directed at understanding this problem has yielded new relatively non-toxic trichothecenes.[6,7] In this paper we report the structures, spectral data and biological activities of three new toxic metabolites: an unusually oxidized trichothecene, a novel modified trichothecene, and the first reported trichothecene with a propionate ester group.



1



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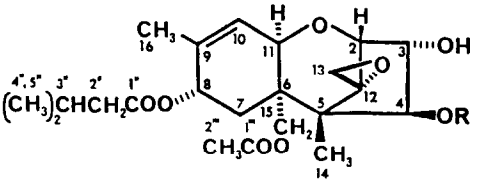

3 R = $COCH_2CH_3$

4 R = $COCH_3$

F. sporotrichioides MC-72083 was cultured[6,8], subjected to extraction and chromatography as reported earlier.[6]

8β-Hydroxytrichothecene **1**, $C_{15}H_{22}O_3$ (m/z 250.150, calc. 250.156), oil, has a band in its i.r. spectrum consistent with the presence of hydroxyl (film 3352 cm^{-1}). The ^1H n.m.r. (Table) shows

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the presence of a vinylic (δ 1.77, 3H, bs) and two tertiary methyls (δ 0.96, 6H, s) as well as characteristic splitting patterns of the 12,13-epoxide methylene protons (δ 3.06, 3.37, both d, J = 4.1 Hz). Two dimensional n.m.r. techniques (COSY[9], NOESY[10]) were performed to place the hydroxyl group observed in the i.r. at C-8 and assign all protons. The relative stereochemistry of the C-8 hydroxyl was determined to be β, based on the large couplings observed from 8-H to 7-Ha (5.5 Hz) and 7-Hb (9.3 Hz), and the small coupling to 11-H (< 1 Hz). This is the first example of a naturally occurring trichothecene with the depicted C-8 stereochemistry. The heteronuclear techniques INAPT[11] and SPT[12] were performed to verify the structure as depicted and assign all the carbons (Table) using ^{1}H-^{13}C spin-spin couplings over one, two and three bonds.

FS-1 **2**, $C_{15}H_{22}O_3$ (m/z 250.150, calc. 250.156), oil, has bands in its i.r. spectrum suggesting the presence of an enone (film, 1684 cm^{-1}) as well as an hydroxyl functionality (film, 3383 cm^{-1}). The u.v. spectrum supported either a cyclopentenone or a cyclohexenone moiety (MeOH, λ_{max} 234, ε 7,500). A solution i.r. spectrum of **2** led us to conclude that the enone was probably a cyclopentenone (CCl_4, 1716 cm^{-1}), although further evidence was clearly necessary. The ^{1}H n.m.r. (Table) indicated the presence of a vinylic (δ 1.68, 3H, bs) and two tertiary methyls (δ 0.87, 3H, s; δ 1.32, 3H, s), an isolated methylene next to oxygen (δ 4.41, 4.60, 2H, J_{AB} = 16.5Hz) and two vinyl protons (δ 5.12, 1H, bs; δ 6.32, 1H, t, J = 1.5Hz). Data from COSY, NOESY[10] and DNOES (difference n.O.e. spectroscopy) allowed proton assignments to be made on the tentative structure **2**. The INAPT experiments verified structure **2** as shown and allowed assignment of all the carbons using long-range couplings: 2-H coupled to C-3, C-4, C-5, C-12 and C-13; 4-Ha coupled to C-2, C-5 and C-12, 4-Hb coupled to C-2, C-5, C-6 and C-12; 10-H coupled to C-6, C-8 and C-16; H-11 coupled to C-5, C-6, C-9, C-10 and C-15; H-13b coupled to C-2 and C-12; H-14 coupled to C-4, C-5 and C-6; H-15 coupled to C-5, C-6, C-7 and C-11.

The relative stereochemistry of **2** was determined on the basis of n.O.e.'s obtained from NOESY and DNOES. The key n.O.e.'s were between 4-Ha and 14-H (methyl), 4-Hb to 15-H (methyl), 11-H to 14-H (methyl) with <u>no</u> n.O.e. observed between 11-H and 15-H (methyl) by either technique. This established a <u>trans</u> relationship between 11-H and 15-H (methyl). The n.O.e.'s observed across the C-5/C-6 single bond indicated above also tie the two rings together with the relative stereochemistry as depicted. The absolute stereochemistry is suggested using the inverse helicity rule for cyclopentenones, regarding the signs of the Cotton effects observed in the c.d. spectrum (MeOH, $n\rightarrow\pi^*$, 310 nm, neg; $\pi\rightarrow\pi^*$, 254 nm, pos).[13] This is consistent with C-5 and C-6 having the same absolute stereochemistry[14] as all of the previously isolated trichothecenes but with inversion at C-11.

4-Propanoyl HT-2 **3**, $C_{25}H_{36}O_9$ (CIMS, m/z 470.236, calc. 470.235), white needles, m.p. 141-142°C, has a band in its i.r. spectrum indicative of hydroxyl (film, 3430 cm^{-1}) as well as ester (film, 1741, 1730 cm^{-1}) functionalites. The molecular formula indicated that **3** was a homologue of T-2 toxin **4**[2]. The ^{1}H n.m.r. spectrum of **3** was very similiar to that of **4**, with a few significant differences. Additional peaks in the proton spectrum of **3** at δ2.43 (2H, q, J = 7.5Hz) and δ 1.17(3H, t, J=7.5Hz) indicative of a propanoate group, as well as loss of an acetate methyl group strongly suggested that either the C-4 or C-15 acetate group in T-2 was replaced by a propanoate group. The other difference was a small chemical shift change in

H-4 which is suggestive that the C-4 acetate present in T-2 has been replaced by the propanoate. The ^{13}C n.m.r. spectrum confirmed the presence of the propanoate group by its characteristic chemical shifts (C-1', δ 176.2, s; C-2', δ 27.8, t; C-3', δ 9.0, q). The INAPT technique was used to establish that C-4 was indeed the carbon bearing the propanoate, and not C-15. Irradiation of both 4-H and 8-H simultaneously (due to their close chemical shifts) saw enhancement through polarization transfer to the propanoate carbonyl carbon C-1' as well as other enhancements consistent with **3** as shown.

Preliminary in vitro studies involving cytotoxicity[15] indicate that **1**,**2** and **3** are all less toxic than T-2 toxin.[16]

Table. ^{1}H (300 MHz) and ^{13}C (75 MHz) Assignments of 8β-Hydroxytrichothecene **1**, FS-1 **2**, and 4-Propanoyl HT-2 **3** ($CDCl_3$).

	1		**2**		**3**	
Atom	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
2	3.65 d (3.9)	81.6 d	6.32 t (1.5)	129.7 d	3.70 d (4.9)	78.7 d
3a	1.91 m	30.2 t		207.6 s	4.13 m	78.5 d
3b	1.57 dd (13.0, 7.0)					
4a	2.78 dd (13.0, 7.0)	36.8 t	2.95 d (18.0)	50.1 t	5.28 d (2.5)	84.5 d
4b	1.68 dd (13.6, 7.0)		2.09 d (18.0)			
5		46.8 s		52.8 s		48.4 s
6		44.8 s		41.9 s		42.9 s
7a	2.03 dd (12.8, 5.5)	39.9 t	1.99 m	29.8 t	2.38 dd	27.6 t
7b	1.15 dd (12.5, 9.3)		1.83 m		1.90 d (15.2)	
8a	4.12 m	69.2 d	1.48-1.6 m	27.3 t	5.29 d (3.5)	68.0 d
8b			1.48-1.6 m			
9		136.2 s		136.4 s		136.3 s
10	5.27 m	128.9 d	5.12 bs	124.9 d	5.8 d (5.4)	123.7 d
11	4.66 m	71.6 d	4.27 bs	71.2 d	4.35 d (5.7)	67.3 d
12		69.1 s		186.9 s		64.3 s
13a	3.37 d (4.1)	49.3 t	4.60 d (16.5)	61.1 t	3.06 d (3.9)	47.2 t
13b	3.06 d (4.1)		4.41 d (16.5)		2.80 d (3.9)	
14	0.96 s	20.4 q	1.34 s	21.1 q	0.81 s	6.9 q
15a	0.96 s	13.5 q	0.87 s	12.9 q	4.29 ABq (12.6)	64.6 t
15b					4.06 ABq (12.6)	
16	1.77 s	18.2 q	1.68 br s	22.5 q	1.75 s	20.4 q
1'						176.2 s
2'					2.43 q (7.5)	27.8 t
3'					1.17 t (7.5)	9.0 q
1"						172.7 s
2"					2.05-2.17 m	43.6 t
3"					2.05-2.17 m	25.8 d
4"					0.96 m	22.4 q
5"					0.96 m	22.4 q
1'''						170.1 s
2'''					2.03 s	21.1 q

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