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NOVEL TRICHOTHECENES FROM *Fusarium sporotrichioides*

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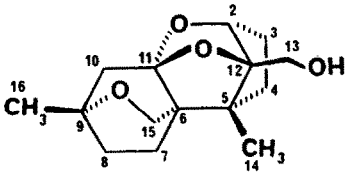
Abstract: Isolation and structural elucidation of two novel trichothecene mycotoxins sporol **1** and sporotrichiol **2** from *Fusarium sporotrichioides* MC-72083 are reported. The new structures were assigned using COSY, NOESY, Selective INEPT and other spectroscopic techniques.

The trichothecenes are a well known class of sesquiterpenoid mycotoxins produced by various genera of fungi, in particular *Fusarium*[1]. Widely occurring, the fungal metabolites are linked to toxicoses in the United States, Japan, Europe and the USSR[2].



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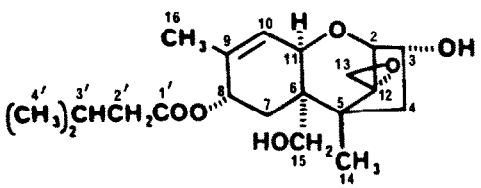

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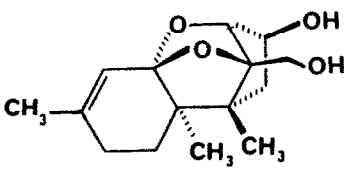

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F. sporotrichioides MC-72083 was cultured[3] on corn medium at 10°C for 21 days. Extraction of the 85:15 $CHCl_3$/acetone solubles, followed by florosil column chromatography (2:1 benzene/hexane, CH_2Cl_2, 95:5 $CHCl_3/CH_3OH$) gave an oil highly enriched in trichothecenes. The prep-HPLC (normal phase SiO_2 :benzene/acetone) followed by prep-RPTLC yielded ten trichothecenes (Table 1), two of which are unreported and novel (**1** and **2**).

Sporol **1**, $C_{15}H_{22}O_4$ (m/z 266.152, calc 266.152), oil, has a band in its i.r. spectrum consistent with the presence of hydroxyl (film, 3422 cm^{-1}). The ^1H n.m.r. ($CDCl_3$, 300 MHz) shows the presence of two tertiary methyls (δ 1.10, 1.15) as well as two isolated methylenes next to oxygen (δ 3.71, 3.84; δ 3.79, 4.30). The upfield methylene was shown to be coupled to an hydroxyl proton by deuterium exchange (D_2O). The ^{13}C n.m.r. ($CDCl_3$, 62.9 MHz) spectrum indicated that **1**

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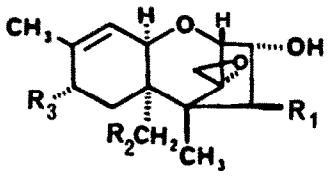
is related to sambucinol **3**[4]. There were significant differences, and two-dimensional n.m.r. experiments (COSY, NOESY) were performed to clarify the structure. Data from COSY[5] and NOESY[6] (Figure) allowed proton assignments to be made (Table 2) and determined the relative stereochemistry to be as depicted. Selective INEPT[7] (also called INAPT[8]) experiments verified the ether linkage, using long-range ^{1}H-^{13}C spin-spin couplings: 15-Ha(δ 3.79) coupled to C-10(δ 44.5) and C-11 (δ 106.8); 15-Hb(δ 4.30) coupled to C-9 (δ 70.3), C-7(δ 25.0) and C-5 (δ 46.8).

Table 1. 12,13-Epoxytrichothecenes Isolated from Fusarium sporotrichioides MC-72083

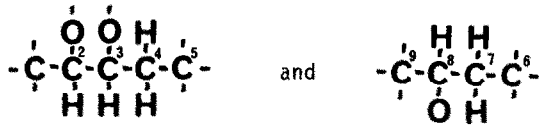


Name	R_1	R_2	R_3
T-2[a]	OAc	OAc	$OOCCH_2CH(CH_3)_2$
HT-2[a]	OH	OAc	$OOCCH_2CH(CH_3)_2$
Sporotrichiol **2**	H	OH	$OOCCH_2CH(CH_3)_2$
NT-1[a]	OAc	OH	OAc
Neosolaniol[a]	OAc	OAc	OH
8-Acetoxy T-2 tetraol[b]	OH	OH	OAc
4-Deacetylneosolaniol	OH	OAc	OH
T-2 tetraol[a]	OH	OH	OH
DAS[a]	OAc	OAc	H

[a]previously reported as occurring in F. sporotrichioides[1,2]
[b]details will be reported elsewhere

Sporotrichiol **2**, $C_{20}H_{30}O_6$ (m/z 366.204; calc 306.206), oil, has bands in its i.r. spectrum suggesting the presence of ester (film, 1726 cm^{-1}) as well as hydroxyl functionality (film, 3422 cm^{-1}). The ^{1}H n.m.r. showed the characteristic splitting pattern of the 12,13-epoxide methylene protons (δ 2.87, 3.06, both d,J = 4.1 Hz) as well as two upfield methyl signals

(δ 0.87, s; δ 0.95, d, J = 7Hz,), a vinylic methyl (bs, δ 1.75) and an isolated methylene next to oxygen (δ 3.53, 3.70, J_{AB} = 12.7 Hz). From COSY (See Table 2) the following isolated structural fragments were obtained:



The 8-H methine (δ 5.50) was also found to be coupled (COSY) to the 16-H vinylic methyl (δ 1.75), as well as long range heteronuclearly coupled (Selective INEPT[7]) to the ester carbonyl (δ 171.9, C-1'), verifying the location of the ester at C-8. Also, after addition of D_2O, the protons at δ 4.47 (3-H) and δ 3.53 (15-Ha) sharpened, indicating the presence of hydroxyl at C-3 and C-15. NOESY[6] experiments allowed the rest of the molecule to be defined, with the relative stereochemistry shown (Figure). It should be noted that sporotrichiol **2** is the first reported trichothecene to have oxidation at C-8 with no oxidation at C-4. Preliminary in vitro studies involving cytotoxicity[9] indicate that **1** and **2** are less toxic than T-2 toxin.[10]

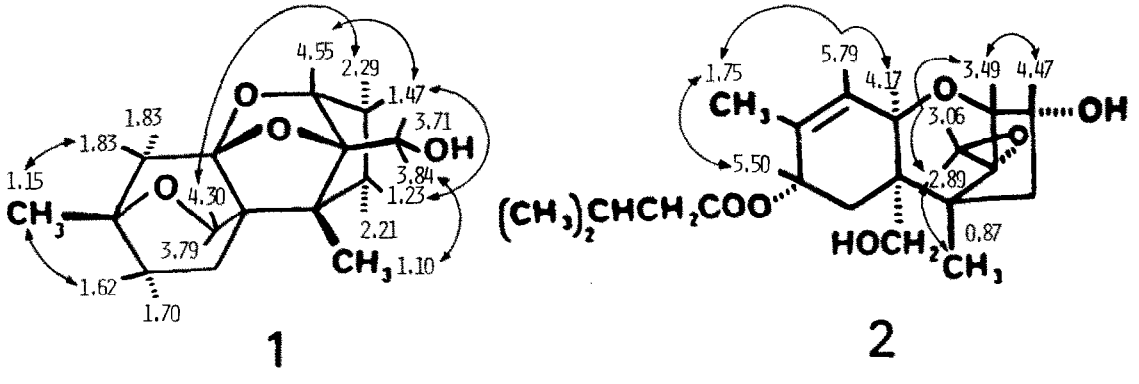


Figure. NOE's obtained from NOESY for sporol **1** and sporotrichiol **2**.

Table 2. ^{1}H and ^{13}C n.m.r Assignments of Sporol **1**, Sporotrichiol **2**, and Sambucinol **3** ($CDCl_3$)[4].

	1		**2**		**3**	
Atom	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
2	4.55d	76.3 d	3.49d	79.6d	3.92	80.9d
3	1.47,2.29m	41.4 t	4.49m	69.1d	4.23dd	72.7d
4	1.23,2.21m	32.0 t	2.05-2.25m	42.1t	2.59dd	46.3t
5	-	46.6 s	-	45.8s	-	50.5s
6	-	47.3 s	-	43.3s	-	48.3s
7	1.75,1.81m	25.0 t	1.96d,2.30dd	26.8t	1.3-2.2m	29.4t
8	1.62,1.70m	41.2 t	5.50d	68.4d	-	30.4t
9	-	70.3 s	-	135.7s	-	144.1s
10	1.83bs	44.5 t	5.79d	125.4d	5.4bs	118.3d
11	-	106.5 s	4.17d	68.1d	-	108.0s
12	-	91.5 s	-	65.1s	-	95.1s
13	3.71,3.84ABq	64.4 t	2.57,3.06dd	48.5t	4.07s	59.6t
14	1.10s	15.4 q	0.87s	12.4q	0.83s	15.2q
15	3.79,4.30ABq	68.4 t	3.53,3.70ABq	63.1t	1.07s	16.7q
16	1.15s	25.7 q	1.75s	20.4q	1.76bs	22.8q
1'			-	171.9s		
2'			2.21m	43.7t		
3'			2.05-2.30m	25.7d		
4'			0.95d	22.3q		

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