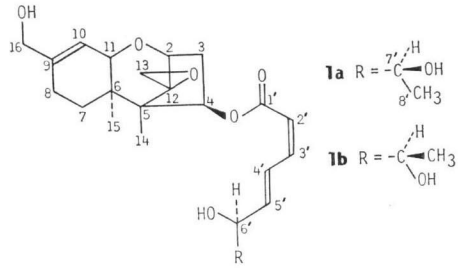
NEW TRICHOVERROIDS FROM *MYROTHECIUM VERRUCARIA*: 16-HYDROXYTRICHODERMADIENEDIOLS

Sir:

We wish to report the isolation of a new set of epimeric trichothecenes from a large scale fermentation of *Myrothecium verrucaria* (ATCC 24571).[1] These new antibiotics, 16-hydroxytrichodermadienediols A and B (**1**) are the first reported naturally occurring trichothecenes in which the C-16 methyl group is substituted, although this position has been hydroxylated by chemical[2] and microbial[3] modifications. Compounds **1a** and **1b** are trichoverroids, a new class of trichothecenes, which have been shown[1,4] to lie along the biosynthetic trail from the simple trichothecenes[5] (*e.g.* T-2 toxin, nivalenol, diacetoxyscirpenol, *etc.*) to the macrocyclic trichothecenes (*e.g.* verrucarins and roridins).[6,7]



The processing of a large scale fermentation (757 liters) of *M. verrucaria* was reported elsewhere.[1] From the ethyl acetate extract of the fermentation beer, and by a series of partition and adsorption column chromatographies was obtained a fraction that was rich in trichoverrins[1,4] and compounds more polar. The fraction thus obtained was subjected to flash chromatography[8] (SiO_2, MeOH - EtOAc). Trichoverrins A and B were obtained followed by a fraction which contained 16-hydroxytrichoder-

Table 1. ^{13}C and ^{1}H NMR data for 16-hydroxytrichodermadienediols A and B (**1a** and **1b**)[a].

Position	16-Hydroxytrichodermadienediol A (**1a**)			16-Hydroxytrichodermadienediol B (**1b**)		
2	79.3	(3.86 d)	[5.1]	79.3	(3.89 d)	[5.1]
3α	37.0	(2.60 dd)	[7.8, 15.5]	36.9	(2.53 dd)	[7.7, 15.4]
3β		(2.04)			(2.03)	
4	75.0	(5.70 m)		75.6	(5.61 m)	
5	49.4	—		49.4	—	
6	41.0	—		41.0	—	
7	23.5	(2.04 m)		23.5	(2.03 m)	
8	24.2	(2.04 m)		24.2	(2.03 m)	
9	143.1	—		143.1	—	
10	118.8	(~5.70)		118.7	(~5.61)	
11	70.7	(3.70 d)	[5.8]	70.1	(3.63 d)	[5.4]
12	66.0	—		66.1	—	
13	48.0	(3.02 AB)	[3.9]	48.0	(2.95 AB)	[3.9]
14	6.2	(0.75)		6.1	(0.67)	
15	16.1	(0.96)		16.0	(0.89)	
16	66.1	(4.05)		66.0	(3.97 brs)	
1′	166.0	—		166.0	—	
2′	118.7	(5.72 d)	[11.2]	118.3	(5.63 d)	[11.4]
3′	143.5	(6.62 dd)	[11.2, 11.2]	143.6	(6.57 dd)	[11.4, 11.4]
4′	128.0	(7.60 dd)	[11.2, 15.5]	128.1	(7.64 dd)	[11.4, 15.5]
5′	142.1	(6.09 dd)	[5.8, 15.5]	141.3	(6.12 dd)	[15.5, 5.8]
6′	76.6	(4.2 brm)		77.3	(4.2 brm)	
7′	70.2			70.3		
8′	18.9	(1.20 d)	[6.3]	17.8	(1.08 d)	[6.4]

[a] All spectra were taken in deuterio-chloroform solvent. The proton chemical shifts are in parenthesis and $J_{H,H}$ in brackets. Spectra were recorded on a WP-200SY IBM instrument. TMS was used as internal standard (0.0 ppm) and chemical shifts are reported in ppm. ^{13}C Chemical shift assignments were done by comparing proton decoupled spectrum with spectra obtained in an INEPT experiment and by comparison with the literature values for trichodermadienediols A and B.[1]

Table 2. ^{1}H NMR chemical shifts for the acetates of trichodermadienediols A and B[a].

Position	16-Hydroxy-trichodermadienediol triacetate A	16-Hydroxy-trichodermadienediol triacetate B
2	3.85 d (5.1)	3.86 d (5.1)
3α	2.58 dd (7.7, 15.5)	2.55 dd (7.8, 15.4)
4	5.62 dd (7.7, 3.5)	5.64 dd (7.8, 3.5)
5	—	—
6	—	—
7		
8		
9	—	—
10	5.72 d (5.0)	5.74 d (5.2)
11	3.69 d (5.0)	3.69 (5.2)
12	—	—
13	2.99 AB (4.0)	2.98 AB (4.0)
14	0.74	0.73
15	0.96	0.97
16	4.50	4.50
1′	—	—
2′	5.76 d (11.6)	5.77 d (11.4)
3′	6.56 dd (11.6, 11.6)	6.58 dd (11.4, 11.4)
4′	7.60 dd (11.6, 15.5)	7.64 dd (11.4, 15.5)
5′	5.90 dd (6.1, 15.5)	5.94 dd (7.1, 15.5)
6′	5.46 dd (6.1, 6.1)	5.49 ddd (0.5, 3.5, 7.1)
7′	5.09 dq (6.1, 6.1)	5.11 dq (3.5, 6.5)
8′	1.20 d (6.1)	1.22 d (6.5)
C–CH$_3$ (O)	2.05	2.05
C–CH$_3$ (O)	2.08	2.08
C–CH$_3$ (O)	2.16	2.10

[a] Spectra were recorded on WP-200SY IBM instrument in deuterio-chloroform with TMS as internal standard (0.0 ppm). Chemical shifts are reported in ppm and coupling constants in Hz are listed in parenthesis.

madienediols A (**1a**) and B (**1b**). These were separated and isolated by HPLC, employing C-18 reversed phase column (50% methanol - water), with **1b** eluting before **1a**.

The structure assignments for **1a** and **1b** are based on the following data: 16-hydroxytrichodermadienediol A (**1a**): oil $[\alpha]_D^{25}$ $-25\pm0.20°$ (*c* 0.50, $CHCl_3$); λ_{max}^{MeOH} 260 nm (log ε 4.25); mass spectrum (chemical ionization, methane gas reagent) *m/z* 421.2229 (M+H, calcd. 421.2226). The ^{1}H and ^{13}C NMR spectral data for **1a** and **1b** are presented in Table 1. Table 2 presents the ^{1}H NMR data for the acetates. The ^{1}H NMR spectrum of **1a** is similar to that for trichodermadienediol A[1,4] except that in trichodermadienediol A the C-16 methyl appears as a broad singlet (3H) at δ 1.74 while in **1a** this signal is now a two proton broad singlet at δ 4.05. Acetylation (pyridine - Ac_2O) resulted in the formation of a triacetate which suggested the presence of three hydroxyl groups. The broad singlet at δ 4.05 shifts downfield by 0.5 ppm in the acetate. In the ^{13}C spectrum of trichodermadienediol A, C-16 appears at δ 23.2,[1] while in the 16-hydroxyl derivative (**1a**) it appears at δ 66.1 (Table 1). Also a ^{13}C NMR INEPT (Insensitive nucleus enhanced polarization transfer) experiment[10] showed that **1a** has five methylene carbons and thirteen methine and methyl carbons. Trichodermadienediol A has four methylene carbons and fourteen methine and methyl carbons. Based on this and relevant proton NMR data, it is evident that the C-16 methyl group has changed to a methylene due to hydroxylation. That the triol belongs to the A series (*i.e.*, L-*threo*)[1,4] was concluded from the ^{1}H NMR spectrum of the triacetate in which the 7′-H resonates at δ 5.09 as an overlapping doublet of quartets ($J_{7',8'}=J_{6',7'}=6.1$ Hz).

Data for 16-hydroxytrichodermadienediol B (**1b**) are as follows: oil $[\alpha]_D^{25}$ $-19.4\pm0.3°$ (*c* 1.2, $CHCl_3$); λ_{max}^{MeOH} 260 nm (log ε 4.27); mass spectrum (chemical ionization, methane gas reagent) *m/z* 421.2231 (M+H, calcd. 421.2226). The ^{1}H NMR spectrum (Table 1) of 16-hydroxytrichodermadienediol B (**1b**) is very similar to that of its epimer, **1a**. The two epimers may be distinguished by use of the proton spectra of the acetates (Table 2). The D-*erythro* configuration for **1b** was established by the observation of an eight line multiplet ($J_{7',8'}=6.5$ Hz, $J_{6',7'}=3.5$ Hz) at δ 5.11, in the triacetate of **1b**, which is assignable to H-7′.[1,4] The corresponding proton in the A epimer resonates at δ 5.09 and exhibits a five line multiplet with $J_{6',7'}=J_{7',8'}=6.1$ Hz. Furthermore, in the acetates, H-6′ appears as a three line doublet of doublets ($J_{5',6'}=J_{6',7'}=6.1$ Hz) at δ 5.46 for the **1a** epimer, but H-6′ appears as an eight line doublet of doublets of doublets ($J_{4',6'}=0.5$ Hz, $J_{5',6'}=7.1$ Hz, $J_{6',7'}=3.5$ Hz) at δ 5.49 for the B epimer.

Compounds **1a** and **1b** were isolated in only small amounts (<1 mg/liter) from this culture of *M. verrucaria*. However, we have examined a

polar fraction of an extract from a culture of *M. roridum* (strain 514)[6] and find that this organism also produces **1a** and **1b** in addition to several other C-16 hydroxylated congeners. The yields of the C-16 hydroxylated derivatives from this strain of *M. roridum* appear to be appreciably higher than those observed from *M. verrucaria* (ATCC 24571). Although we have no biological activity data for **1**, we anticipate that these compounds, like other trichoverroids,[1,4] will be substantially less bioactive than the macrocyclic trichothecenes.

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