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NMR ASSIGNMENTS OF TERRITREMS A, B, AND C AND THE STRUCTURE OF MB_2, THE MAJOR METABOLITE OF TERRITREM B BY RAT LIVER MICROSOMAL FRACTION

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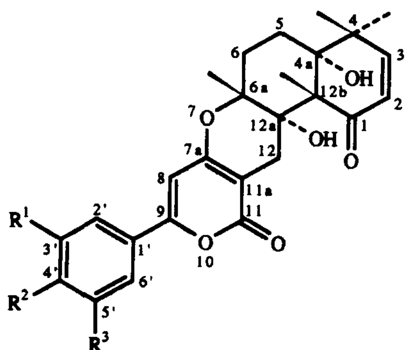
ABSTRACT.—The ^{1}H- and ^{13}C-nmr assignments of territrems A, B, and C [**1–3**] were made by using nOe and 2D nmr techniques. Following the same methods, the structure of MB_2 [**4**], the major product of territrem B incubated with rat liver microsomal fraction, was determined as a hydroxylation product at the pro *S* methyl group of C-4 of territrem B.

Ling and co-workers (1,2) and Yang (3) have reported the isolation of three tremorgenic mycotoxins, territrems A [**1**] ($C_{28}H_{30}O_9$), B [**2**] ($C_{29}H_{34}O_9$), and C [**3**] ($C_{28}H_{32}O_9$), from the rice culture of *Aspergillus terreus*. These three compounds, unlike other known tremorgenic mycotoxins (4), do not contain nitrogen. The similarity of their uv, ir, and mass fragmentation patterns suggested that these compounds possessed a common skeleton. Their structures were partially characterized by ^{1}H nmr, uv, and chemical degradation with H_2O_2 and *O*-methylation (1–3, 5). The chemical structure of territrem B was ultimately determined by X-ray crystallography as (4a*R*,6a*R*,12a*S*,12b*S*)-4a,6,6a,12,12a,12b-hexahydro-4a,12a-dihydroxy-4,4,6a,12b-tetramethyl-9-(3,4,5-trimethoxyphenyl)-4*H*,11*H*-naphtho(2,1-*b*)pyrano(3,4-*e*)pyran-1,11(5*H*)-dione (6). Following this, the structures of territrems A and C were determined as **1** and **3**. In this current study of the physical properties and metabolism of these unusual mycotoxins, the nmr assignments of **1–3** were made. In addition, the structure of MB_2, the major metabolite of territrem B transformed by a microsomal fraction from rat liver (7), was also elucidated.



1 R^1+R^2=-OCH_2O-, R^3=OMe
2 $R^1=R^2=R^3$=OMe
3 $R^1=R^3$=OMe, R^2=OH
4 $R^1=R^2=R^3$=OMe; 4β-CH_2OH
5 $R^1=R^2=R^3$=OMe; 4β-CH_2OAc

The proton signals of territrem B [**2**] in the high field nmr were well resolved (Table 1). The olefinic and aromatic protons were readily assigned by their coupling patterns and chemical shifts. The AB quartets at δ 6.29 and 5.91 (J = 7.6 Hz) were assigned as H-3 and H-2, respectively, due to the electron shielding at C-2. The singlet at δ 7.22 (2 × H) was assigned to the equivalent H-2′ and H-6′ protons. By elimination, the remaining olefinic proton, H-8, was assigned to the δ 6.78 signal. The aliphatic region shows the signals of three methoxys, four methyls, and three methylenes. The three methoxys appearing as two singlets at δ 3.81 (2 × OMe) and 3.91 (1 × OMe) were assigned as 3′,5′-methoxys and 4′-methoxy, respectively. Of the six methylene protons, the sig-

TABLE 1. ^{1}H-nmr Data of Compounds **1**–**4** (δ in ppm, J in Hz).[a]

Proton	Compound			
	2	**1**	**3**	**4**
H-2	5.90 d (10.0)	5.89 d (12.0)	5.80 d (10.0)	6.02 d (12.0)
H-3	6.29 d (10.0)	6.26 d (12.0)	6.28 d (10.0)	6.84 d (12.0)
H-5	1.81–1.90 m	1.83–1.86 m	1.82–1.90 m	1.84–1.92 m
H-6a	1.90 m	1.91 m	1.95 m	1.95 m
H-6b	2.80 m	2.80 m	2.40 m	2.97 m
H-8	6.78 s	6.79 s	6.32 s	6.84 s
H-12α	4.20 d (17.6)	4.26 d (18.0)	3.43 d (18.0)	4.31 d (17.6)
H-12β	3.13 d (17.6)	3.09 d (18.0)	2.80 d (18.0)	3.12 d (17.6)
4α-Me	1.29 s	1.25 s	1.32 s	1.67 s
4β-Me	1.21 s	1.16 s	1.22 s	
4β-CH_2				3.97 d (9.0) 3.62 d (9.0)
6a-Me	1.50 s	1.45 s	1.55 s	1.57 s
12b-Me	1.47 s	1.41 s	1.48 s	1.49 s
H-2′	7.29 s	7.19 br s	7.02 s	7.24 s
H-6′	7.29 s	7.19 br s	7.02 s	7.24 s
3′-OMe	3.81 s		3.92 s	3.78 s
4′-OMe	3.91 s			3.90 s
5′-OMe	3.81 s	3.76 s	3.92 s	3.78 s
OCH_2O		5.97 s		

[a]Compounds **1**, **2**, and **4** were measured in C_5D_5N; **3** was measured in $CDCl_3$.

nals of H-12α and H-12β appeared as an AX system (J = 17.6 Hz) and were assigned from analysis of chemical model and nOe's. The model indicated that H-12α would be much more deshielded than H-12β by the ring current of the α-pyranone ring, thus locating H-12α and H-12β at δ 4.20 and 3.13, respectively. The assignments of H-12 together with the four methyls (δ 1.21, 1.29, 1.47, and 1.50) were also confirmed or made by nOe experiments (Figure 1). The irradiation of the methyl signal at δ 1.50 or 1.47 enhanced the doublet at δ 3.13. The latter irradiation at δ 1.47 also enhanced the methyl singlet at δ 1.21. These data thus designated the chemical shifts of 4b-, 6a-, and 12b-methyls at 1.21, 1.50, 1.47, respectively, and the shifts of H-12β at 3.13 and H-12α at 4.20. By correlation with the ^{1}H-nmr data of territrem B, the complete ^{1}H-nmr assignments of territrems A and C are listed in Table 1. Among these, H-6 and H-5 were assigned by a hetero-COSY experiment.

The ^{13}C-nmr assignments of territrem B were made by a hetero-COSY and hetero long-range COSY experiments

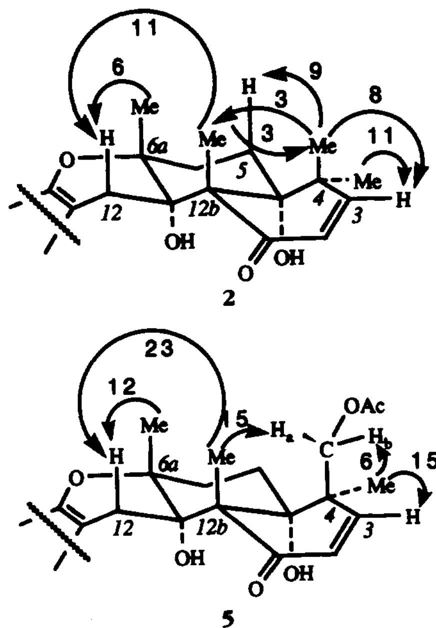


FIGURE 1. NOe's (%) of **2** and **5**.

(Table 2). With the proton signals assigned, those proton-bearing carbons were assigned from the hetero-COSY experiment. The carbon signal at δ 26.2 coupled to H-5 (δ 1.80, 1.85) was assigned to C-5. The proton signal at δ 2.80 was directly coupled to C-6 (δ 29.5) and therefore is one of the H-6's. The other H-6 partially overlapped with H-5 (δ 1.80, 1.85). The quaternary carbons were assigned from a hetero long-range COSY experiment optimized for J_{CH} = 10 Hz. The signals of oxygenated quaternary carbons, C-6a (δ 81.5), C-4a (δ 79.5), and C-12a (δ 76.2), were assigned from their long-range couplings to 6a-CH_3 (δ 1.50) (C-6a, C-12b), 12b-CH_3 (δ 1.47) (C-12b, C-4a), 4β-CH_3 (δ 1.21) (C-4a), and 4α-CH_3 (δ 1.29) (C-4a). The assignments of C-4 at δ 42.8 and C-12b at δ 56.5 were made by their two-bond couplings with 4,4-dimethyl and 12b-methyl, respectively. The chemical shifts of C-7a (δ 163.1) and C-9 (δ 158.3) were distinguished via their couplings to H-8 (C-7a and C-9) and H-2′ (or H-6′) (C-9). The overlapping signals of C-8 and C-11a at δ 98.4 were clarified from the hetero-COSY spectrum for C-8 and the hetero long-range COSY spectrum in which C-11a is coupled to H-12α and H-12β (δ 4.20

TABLE 2. ^{13}C-nmr Data of Compounds **1–4** in C_5D_5N (δ in ppm, m).[a]

Carbon	Compound				Hetero long-range COSY data of **2**	
	1	**2**	**3**	**4**	δ_c	δ_H
C-1	202.2 s	202.1 s	202.7 s	202.2 s	202.1	1.47 (12b-Me), 6.29 (H-3)
C-2	124.0 d	124.2 d	122.0 d	125.0 d		
C-3	153.1 d	153.1 d	153.6 d	154.1 d	153.1	1.21, 1.29 (4-Me's)
C-4	42.8 s	42.8 s	42.8 s	49.1 s	42.8	1.21, 1.29 (4-Me's)
C-4a	79.4 s	79.5 s	80.1 s	79.7 s	79.5	1.21, 1.29 (4-Me's), 1.47 (12b-Me)
C-5	26.2 t	26.2 t	26.0 t	26.5 t		
C-6	29.4 t	29.5 t	29.4 t	29.4 t	29.5	1.50 (6a-Me)
C-6a	81.5 s	81.5 s	81.8 s	81.6 s	81.5	1.50 (6a-Me)
C-7a	163.2 s	163.1 s	164.0 s	163.3 s	163.1	6.78 (H-8)
C-8	98.0 d	98.4 d	97.7 d	98.7 d		
C-9	158.1 s	158.3 s	159.1 s	158.4 s	158.3	6.78 (H-8), 7.29 (H-2′, -6′)
C-11	163.9 s	163.8 s	166.1 s	164.2 s		
C-11a	98.0 s	98.4 s	97.2 s	98.6 s	98.4	3.13, 4.20 (H-12), 6.78 (H-8)
C-12	27.5 t	27.6 t	27.5 t	27.8 t		
C-12a	76.2 s	76.2 s	76.2 s	76.3 s	76.2	1.47 (12b-Me), 1.50 (6a-Me)
C-12b	56.7 s	56.5 s	56.5 s	56.7 s	56.5	1.47 (12b-Me)
4α-Me	25.8 q	25.9 q	25.8 q	20.3 q		
4β-Me	23.9 q	24.0 q	23.9 q			
4β-CH_2				65.8 t		
6a-Me	23.6 q	23.7 q	23.8 q	23.8 q		
12b-Me	22.0 q	22.1 q	22.1 q	22.1 q		
C-1′	123.2 s	127.6 s	122.0 s	127.7 s	127.6	6.78 (H-8), 7.29 (H-2′, -6′)
C-2′	106.5 d	103.7 d	103.0 d	103.9 d		
C-3′	150.0 s	154.3 s	153.6 s	154.4 s	154.3	3.81 (3′-OMe), 7.29 (H-2′)
C-4′	139.1 s	141.3 s	140.3 s	141.3 s	141.3	3.91 (4′-OMe), 7.29 (H-2′, -6′)
C-5′	144.4 s	154.3 s	153.6 s	154.4 s	154.3	3.81 (5′-OMe), 7.29 (H-6′)
C-6′	100.1 d	103.7 d	103.0 d	103.9 d		
3′-OMe		56.4 q	56.7 q	56.6 q		
4′-OMe		60.8 q		60.9 q		
5′-OMe	56.5 q	56.4 q	56.7 q	56.6 q		
OCH_2O	102.7 t					

[a]Multiplicities were obtained from DEPT experiment.

and 3.13) and H-8 (δ 6.78). The chemical shift of C-6 (δ 29.5) was also confirmed by its three-bond coupling to 6a-methyl (δ 1.50). The remaining assignments were clearly made from their couplings as shown in Table 2. By correlation with the ^{13}C-nmr data of **2**, the ^{13}C-nmr spectra of **1** and **3** were also assigned and listed in Table 2.

MB_2 [**4**] is a major metabolite of territrem B incubated with the S_9 fraction of rat liver (7). Its hrms shows the molecular ion at *m/z* 542.2126 for the formula of $C_{29}H_{34}O_{10}$ (calcd 542.2152), one more oxygen than that of territrem B. Its ^{1}H-nmr spectrum showed only three methyl signals, one fewer than that of territrem B. Its ^{13}C-nmr spectrum indicated one additional oxygenated methylene at δ 65.8 and lack of a methyl signal. Except for these differences, MB_2 and territrem B showed very similar spectral patterns including nmr, mass, and uv spectra. These spectral differences indicated that MB_2 is simply territrem B hydroxylated at one of four methyl groups. Acetylation yielded a monoacetate product **5** (Me of OAc at δ 2.11). NOe's of **4** indicated that the irradiation of the methyl singlet at δ 1.57 or δ 1.49 enhanced the doublet signal of H-12β (δ 3.12, $J=17.6$ Hz). Another irradiation of the remaining methyl singlet (δ 1.67) enhanced only H-3 (δ 6.84, d, $J=12$ Hz). These results located 6a-, 12b-, and 4-methyls at δ 1.57, 1.49, and 1.67, respectively. Since no nOe was observed between 4-CH_3 and 12b-CH_3, the methyl group at C-4 must be α-oriented by correlation with the nOe's of territrem B. Consequently, MB_2 is 4β-hydroxymethyl-4β-demethyl territrem B. This structure was confirmed from nOe's of **5** (Figure 1). Irradiation of the methyl singlet at δ 1.53 or δ 1.43 enhanced the doublet signal of H-12β (δ 2.81, $J=17.8$ Hz). The irradiation at the singlet at δ 1.53 also enhanced a part of AB quartets at δ 4.50 ($J=11.0$ Hz). These data located 12b-Me, 6a-Me, and 4β-CH_a at δ 1.53, 1.43, and 4.50, respectively, and confirmed that the hydroxymethyl group of **4** is 4β-oriented. Another irradiation of the remaining methyl singlet (δ 1.25, 4α-Me) enhanced the remaining part of AB quartets at δ 4.08 (4β-CH_b), and H-3 (δ 6.38, d, $J=10.4$ Hz) further supported this structure assignment.

By comparison with **2**, the ^{1}H- and ^{13}C-nmr assignments of **4** were also made and are listed in Tables 1 and 2, respectively.

EXPERIMENTAL

General experimental procedures.—Optical rotations were measured on a Jasco DIP-181 Digital Polarimeter. Nmr spectra were recorded on Bruker AC 80 or Bruker AM 300 spectrometers using $CDCl_3$ (δ 7.24 for ^{1}H) or C_5D_5N (δ 8.71 for ^{1}H and 149.9 for ^{13}C) as references. Uv spectra were measured with Jasco Uvidec-1 spectrophotometer. CD spectra were measured with Jasco-J 500A spectrophotometer in MeOH. Ei mass spectra were measured with a Jeol JMX-HX 110 mass spectrometer at 70 eV. 2D nmr spectra and 1D nOe were measured with Bruker's standard pulse programs. The settings of 1D nOe are: 6 mg sample in 0.5 ml D-solvent (deoxygenated with N_2), NS = 16 per experiment, NE = 50, DS = 2, D_1 = 8 sec, D_2 = 5 msec, DP = 60L, 90 degree pulse is applied for data acquisition at 298K. In hetero-COSY and hetero long-range COSY (65 mg in 0.5 ml C_5D_5N), a 1-sec delay was allowed between each scan, and the coupling constant was optimized for $J=125$ Hz and 10.0 Hz, respectively. The 2D correlation maps consisted of 512 × 1K data points per spectrum, each composed of 320 transients.

Extraction and isolation.—Territrems were isolated from $CHCl_3$ extracts of rice cultures of *A. terreus* 23-1 incubated at 28°–30° for 21 days as stationary cultures (1–3). Territrem B has the following physical properties: uv (MeOH, log ε) λ max 331 nm (4.16) and 217 nm (4.40); CD (MeOH) $\Delta\epsilon_{233}$ + 0.55; eims *m/z* (rel. int.) $[M]^+$ 526 (39), $[M-H_2O]^+$ 508 (67), $[M-H_2O-Me]^+$ 493 (100), 359 (20), 345 (10), 195 (52); ^{1}H nmr see Table 1; ^{13}C nmr see Table 2.

MB_2 [**4**].—MB_2 was isolated from the reaction mixture of territrem B, S_9 fraction of rat liver, NAD, glucose 6-phosphate, $MgCl_2$, and KCl (7). It has the following physical properties: $[\alpha]^{25}D$ +69° ($c=0.2$, MeOH); uv (MeOH, log ε) 331 nm (4.14) and 218 nm (4.44); CD (MeOH) $\Delta\epsilon_{233}$ + 0.56; nOe data in C_5D_5N 4α-Me to H-3 13%, 6a-Me to H-12β 13%, 12b-Me

to H-12β 5%; eims *m/z* (rel. int.) $[M]^+$ 542 (5), $[M-H_2O]^+$ 524 (15), $[M-H_2O-Me]^+$ 509 (18), 359 (45), 345 (32), 195 (100); hreims *m/z* $[M]^+$ 542.2126 (calcd for $C_{29}H_{34}O_{10}$, 542.2152); ^1H nmr see Table 1; ^{13}C nmr see Table 2.

MB_2 ACETATE [**5**].—The reaction mixture of MB_2 (11.0 mg), Ac_2O (1.0 ml), and pyridine (0.3 ml) was kept at room temperature for 48 h. After evaporation under vacuum the residue was purified via a 3 g Si gel column eluted with 0.5 and 1% MeOH in $CHCl_3$ to give 9.0 mg of MB_2 acetate: ^1H nmr ($CDCl_3$) 6.98 (s, H-2′, -6′), 6.38 (d, J = 10.4 Hz, H-3), 6.34 (s, H-8), 5.88 (d, J = 10.4 Hz, H-2), 4.50 (d, J = 11.0 Hz, 4β-CH_a), 4.08 (d, J = 11.0 Hz, 4β-CH_b), 3.88 (s, 3′,4′,5′-OMe), 3.39 (d, J = 17.8 Hz, H-12α), 2.81 (d, J = 17.8 Hz, H-12β), 2.11 (s, Me of OAc), 1.53 (s, 12b-Me), 1.43 (s, 6a-Me), 1.25 (s, 4α-Me); eims *m/z* (rel. int.) $[M]^+$ 584 (48) (calcd for $C_{31}H_{36}O_{11}$), 566 (44), 548 (29), 524 (20), 522 (20), 476 (20), 276 (16), 210 (100), 195 (53).

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