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MYROTOXINS: A NEW CLASS OF MACROCYCLIC TRICHOTHECENES

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Abstract: An isolate of Myrothecium roridum which is a pathogen on tomatoes in Texas produces a new class of C27 macrocyclic trichothecenes which contains a nonconjugated diene system that includes a vinyl ether group.

During the course of our screening of plant pathogenic strains of Myrothecium fungi for antibiotic production,[1] we received an isolate of Myrothecium roridum which is a pathogen on tomatoes in Texas.[2] Growth of this organism in submerged liquid cultures[3] did appear to yield macrocylic trichothecenes,[4-6] but the yields were poor. However, when grown on rice,[7] this fungus yielded two new macrocyclic trichothecenes in good yield (ca. 50 mg/200 g of rice for each compound). These compounds were isolated from a methanol extract of the rice by filtration chromatography and preparative TLC on a chromatotron (Model 7924, Harrison Research Laboratories, Palo Alto, CA). Base hydrolysis of myrotoxin A [mp 220-222°C; $[\alpha]_D^{25°}$ 122.0 (C 0.50, CH_2Cl_2); HRMS-FAB/glycerol/Xe 501.2154 (calcd. for $C_{27}H_{32}O_9$ + H 501.2125)] gave verrucarol, whereas hydrolysis of myrotoxin B [mp 195-197°C; $[\alpha]_D^{25°}$ 101.7 (C 0.60, CH_2Cl_2); HRMS-FAB/glycerol/Xe 559.2244 (calcd. for $C_{29}H_{34}O_{11}$ + H 559.2179)] gave 8α-hydroxyverrucarol.[3] Both ^1H and ^{13}C NMR spectra of these compounds made it evident that the myrotoxins are neither roridins or verrucarins, the usual macrocylic trichothecenes produced by Myrothecium species.[8] The most striking features of the ^1H NMR spectra are the signals at δ 6.6 and δ 5.9 which are due to the H9' and H10' vinyl hydrogens, respectively. In this regard, these spectra resemble that of vertisporin,[9] the only other reported naturally occurring macrocyclic trichothecene lacking the 7',8',9',10'-dienic system. But unlike vertisporin, the myrotoxins contain 27 carbon atoms rather than 29 carbon atoms as in vertisporin, the satratoxins,[10] and the roridins.[4]

Myrotoxins A and B each contain one secondary hydroxyl group which is attached to a CH group whose proton appears as a sharp singlet (after D_2O exchange) at ca. δ 3.7. In addition, the singlet at ca. δ 3.3 in the myrotoxins is typical of the 2'H in compounds con-

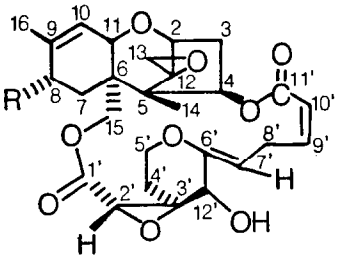
Table 1. ^{13}C and ^{1}H NMR Data for Myrotoxins A and B[a]

Position	Myrotoxin A	Myrotoxin B
2	79.1 (3.85 d) [5.0]	78.9 (3.85 d) [5.2]
3	35.1 (α: 2.48 dd) [15.5, 8.2] (β: 2.17 ddd) [15.5, 5.0, 3.8]	35.0 (α: 2.49 dd) [15.7, 8] (β: 2.17 ddd) [15.7, 5.2, 3.6]
4	74.8 (5.85 dd) [8.2, 3.8]	74.8 (5.83 dd) [8.1, 3.6]
5	49.5	49.5
6	43.2	42.0
7	20.2 (2.0 m)	24.3 (A: 2.1 m; B: 2.07 m)
8	27.4 (2.1 m)	68.1 (5.28 d) [5.2]
9	140.6	136.7
10	118.5 (5.42) [4.2]	123.6 (5.66 d) [5.4]
11	67.4 (3.69) [4.2]	66.8 (3.68 d) [5.4]
12	65.4[b]	65.7[b]
13	47.6 (AB: 3.13, 2.79) [4.0]	47.5 (AB: 3.12, 2.80) [4.0]
14	7.8 (0.80 s)	7.7 (0.79 s)
15	64.2 (AB: 4.68, 3.85) [12.0]	68.1 (AB: 4.89, 3.92) [12.0]
16	23.3 (1.73 s)	21.1 (1.76 s)
1'	164.7	164.5
2'	57.1 (3.36 s)	56.7 (3.32 s)
3'	65.3[b]	65.2[b]
4'	23.5 (A: 2.84 m; B: 1.90 m)	23.2 (A: 2.76 m; B: 1.89 m)
5'	67.2 (A: 4.25 m; B: 4.00 m)	67.1 (A: 4.28 m; B: 3.98 m)
6'	150.9	150.9
7'	109.8 (4.65 dd) [9.8, 2.0]	109.7 (4.65 dd) [9.8, 2.0]
8'	24.3 (A: 4.12 dddd) [14.6, 9.8, 8.8, 1.5] (B: 2.60 ddd) [14.6, 9.0, 2.0]	26.7 (A: 4.11 dddd) [14.6, 9.8, 8.7, 1.6] (B: 2.61 ddd) [14.6, 9.0, 2.0]
9'	148.7 (6.59 ddd) [11.2, 9.0, 8.8]	149.0 (6.60 ddd) [11.2, 9.0, 8.7]
10'	121.9 (5.93 dd) [11.2, 1.5]	121.9 (5.92 dd) [11.2, 1.6]
11'	167.1	166.8
12'	72.1 (3.85 d) [3.6, s after H-D exchange]	72.1 (3.61 d) [3.7, s after H-D exchange]
		20.5 (1.98 s) $\underline{C}H_3CO_2$
		170.8 $CH_3\underline{C}O_2$

a ^{13}C spectra were recorded in $CDCl_3$ on an IBM WP-200SY spectrometer. The ^{1}H spectra were recorded in $CDCl_3$ on a Bruker AM400 spectrometer. The proton chemical shifts are in parentheses and the $J_{H,H}$ in brackets. ^{13}C chemical shift assignments were done by comparing proton decoupled spectra with spectra obtained in an INEPT experiment[11] and by comparison with the literature values for the macrocyclic trichothecenes.[8]

b Assignments may be reversed.

taining a 2',3'-epoxide group; carbon resonances at ca. 57 ppm and 65 ppm also confirm the presence of this epoxide functionality. What is unique in the myrotoxins is a set of vinyl carbon resonances at ca. 151 (C) and 110 (CH) ppm which strongly suggests the presence of a vinyl ether linkage in the macrocyclic portion of the molecule. Complete ^{1}H and ^{13}C NMR data are presented for the myrotoxins in Table 1.



Myrotoxin A: R=H
Myrotoxin B: R=OAc

2D COSY Experiments[11] established all the proton coupling relationships in myrotoxins A and B. In particular, for myrotoxin B the vinyl resonance at δ 4.65 (H 7') is coupled to both H 8'A (δ 4.11) and H 8'B (δ 2.61) which in turn are coupled to H 9' at δ 6.60. It is striking that the diastereotopic protons at C 8' resonate at frequencies which differ by 1.5 ppm. Models suggest that H 8'A (δ 4.11) lies in the deshielding region of the C 11' carbonyl group and is perpendicular to the 9',10' double bond since H 8'A exhibits appreciable allylic coupling (1.6 Hz) with H 10'. A 2D NOESY experiment[11] showed that both H 2' and H 7' gave NOE's with H 12' which establishes the relative stereochemistries for C 2',C 3' and C 6',C7' as shown in the molecular diagram.

Examination of the culture extract has shown that there are several other minor metabolites of similar structure to myrotoxins A and B, but there is no evidence that this isolate produces any trichoverroids[3] or roridin/verrucarin type trichothecenes. Thus, the biosynthetic origin of the myrotoxins, cf. the roridins, is unclear. In particular, the origin of the unconjugated diene system is an interesting question.

The myrotoxins exhibit appreciable cytotoxicity against L-1200 cells with myrotoxin A having an ID_{50} of 0.0005 μg/ml and myrotoxin B having an ID_{50} of 0.0017 μg/ml. These data are comparable to similar cytotoxicity data for vertisporin.[9] Complete reduction of the 7', 8',9',10'-diene system results in appreciable loss of toxicity in the macrocyclic

trichothecenes,[4] whereas both vertisporin and the myrotoxins exhibit cytotoxicities of the same order as the conjugated dienic verrucarins and roridins.

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