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THE COMPLETE SPECTRAL ASSIGNMENT OF DIDEMNIN B AND NORDIDEMNIN B

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Summary: Complete spectral assignments for the cyclic peptides nordidemnin B and didemnin B, isolated from *Tridemnum solidum*, collected at Guadaloupe Island in the Caribbean, are presented.

Didemnid tunicates, particulary *Lissoclinum patella* and *Trididemnum solidum*, have proven to be a rich source of novel, biologically active peptides.[2] The didemnins, first reported in 1981 by Rinehart[3], were isolated from *T. solidum* collected in the Caribbean and exhibit a wide variety of biological responses including *in vitro* and *in vivo* anti-viral, and anticancer activity.[4] Didemnin B (**1**) is currently in Phase II clinical trials as a potential new cancer chemotherapeutic agent.[5] Didemnin B also demonstrated impressive immunosuppressive activity in the Simonsen host vs graft assay[6] as well as the ability to prolong rat heart allografts *in vivo*.[7] In 1987, the structure of didemnin B was revised to **2** based on total synthesis[8] and single crystal x-ray diffraction studies.[9] This revision involved the replacement of the amino acid statine with iso-statine.

We now wish to report the structure elucidation of nordidemnin B (**3**), a lower methylene homologue of **2**, and the complete ^{1}H and ^{13}C NMR spectral assignments for both **2** and **3**. The two peptides were isolated[10] from *T. solidum* collected at Guadaloupe Island[11]. Preliminary spectral data, including FTIR, ^{1}H and ^{13}C NMR and FABMS, confirmed that one of the metabolites was didemnin B. The second metabolite exhibited very similar spectral data to didemnin B but differed by 14 amu's suggesting the loss of a methyl or methylene group.[12] The close structural relationship between the two peptides, coupled with the absence of detailed ^{1}H and ^{13}C NMR assignments for didemnin B in the literature led us to undertake an NMR study of both peptides.

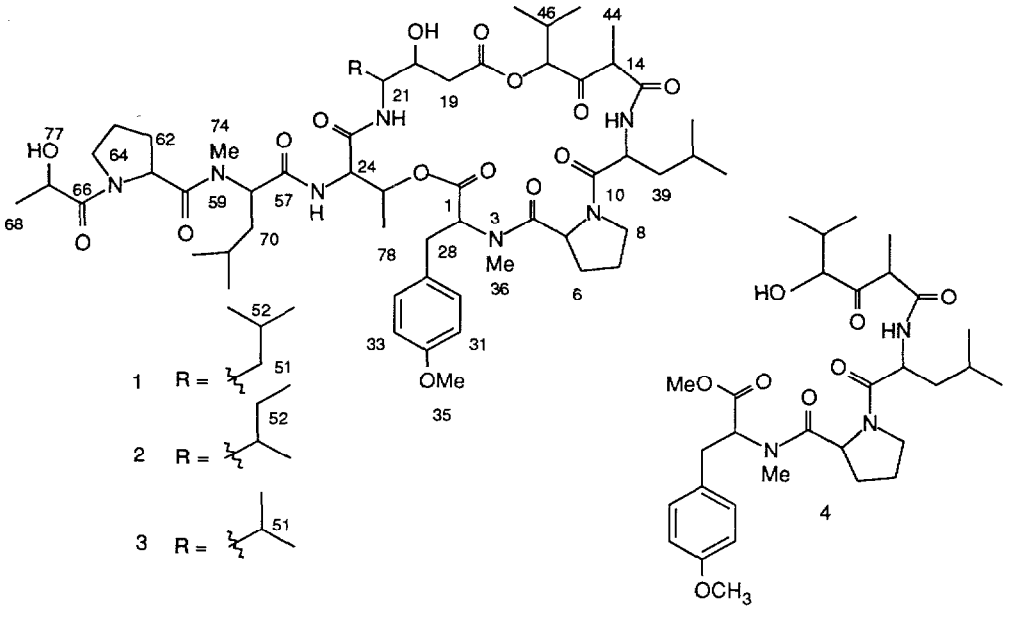
The ^{1}H NMR spectrum of didemnin B (**2**) $C_{57}H_{89}N_7O_{15}$ obtained at 500 MHz (C_6D_6) contained three doublet signals above 7.30 ppm which slowly exchanged with D_2O indicating their attachment to a heteroatom, the characteristic AB pattern of a tyrosine amino acid, and 14 methyl bands including one clearly resolved triplet methyl signal, which was part of the iso-statine amino acid.

The ^{13}C NMR spectrum obtained at 125 MHz contained only 53 distinct signals indicating the presence of four degenerate sets of carbons. Two of these are accounted for by the tyrosine unit. The remaining two were determined by HETCOR[13] experiments to be the β and γ carbons of the two proline units.

DEPT[14] and HETCOR experiments established the multiplicities of each carbon and all of the one bond ^{1}H-^{13}C correlations, respectively. A phase-sensitive, double-quantum-filtered COSY experiment (PS,DQF-COSY)[15] defined the spin systems for all of the amino acid partial structures (2 Pro, N,O-diMeTyr, Thr, Leu, N-MeLeu, i-Sta), lactate and hydroxyisovalerylpropionyl (HIP) group, which effectively provided assignments of all the ^{1}H and ^{13}C NMR signals except for the carbonyl and N-methyl carbon signals. The ^{1}H and ^{13}C NMR assignments are summarized in Table 1.

Having established didemnin B as a model, we turned our attention to the new peptide, nordidemnin B (**3**). The HRFABMS of **3** confirmed a molecular formula of $C_{56}H_{87}N_7O_{15}$ (1098.6325; requires 1098.6337 Δ 1.14 mmu). The ^{1}H

NMR spectrum of **3** was almost identical to **2** with only slight differences in the α-amino proton region (4-5 ppm) and the replacement of the triplet methyl signal at δ 1.16 in **2** with a doublet methyl at δ 1.19. As with didemnin B, DEPT and HETCOR experiments established the multiplicities of all carbons and one bond ^{1}H-^{13}C connectivities, respectively. PS,DQF-COSY experiments identified spin systems corresponding to Leu, N-MeLeu,



Thr, N,O-diMeTyr, 2 Pro, Lactate and HIP analogous to didemnin B, plus two additional spin systems corresponding to an iso-propyl group (δ 2.45 1H dt; 1.06 3H d; 1.19 3H d) and a C_3H_6NO unit (δ 7.50 1H d; 4.48 1H m; 4.47 1H m; 2.82 1H exch. m; 3.95, 2.85 2H dd) which failed to show further coupling in the COSY. In contrast, a total correlation spectroscopy experiment (TOCSY or 2D HOHAHA)[16], exhibited cross peaks for coupling between the signals at δ 4.48 and 2.45, defining the amino acid nor-statine. The TOCSY experiment also provided additional proof for the other spin systems observed in the COSY experiment. Furthermore, saponification of nordidemnin B in 1% KOH in MeOH, gave **4**. The FABMS of **4** displayed MCH_4^+ and MNa^+ ions at *m/z* 590 and 612, respectively, plus ions at *m/z* 433, 320 and 223 indicative of sequential loss of HIP, Leu and Pro from the C terminus of **4**, as well as *m/z* 367 corresponding to the tripeptide HIP-Leu-Pro. The identical product was obtained from saponification of didemnin B. The fragment representing the remainder of the peptide was not isolated from either **2** or **3** which probably reflects the instability of the β-hydroxy carbonyl portion of iso-statine and nor-statine units toward base.

The assignments of the carbonyl and N-Me carbon resonances of nordidemnin B are based on long range ^{1}H-^{13}C correlations obtained from three separate experiments: long range HETCOR, COLOC[17], and INAPT[18]. For example, long range correlations observed for C-57 (MeLeu) include correlations to H-58 in the long range HETCOR (J_{NCH}=10 Hz) as well as the COLOC experiment. The ketone of Hip (C15) has correlations to C-44 and C-16 in the both long range HETCOR experiments. The long range correlations observed are summarized with the ^{1}H and ^{13}C assignments in Table 1. Assignment of the carbonyl resonances of didemnin B are based on a long range HETCOR (J_{NCH}=10 Hz) and comparison with nordidemnin B.

atom	^{13}C[a]	^{1}H[a] mult. J=Hz	Nordidemnin B Long Range ^{1}H-^{13}C correl.
1	169.1		H-24[b,e], H-25[b]
2	66.1	3.15 dd J=10.3,4.2	
4	170.0		H-2[b] H-36[b,c,e]
5	56.3	4.13 m	H-6a[b]
6	27.5	a 1.37 m	H-7a[b]
		b 1.25 m	
7	24.6	a 1.51 m	
		b 1.25 m	
8	46.7	a 3.28 m	
		b 3.10 m	
10	170.6		H-11[c,d]
11	49.9	5.12 brt J=9.8	
12		8.15 d J=9.3	
13	169.9		H-44[b,c]
14	50.2	4.70 q J=6.9	
15	204.4		H-44[b,c] H-16[b,d]
16	81.4	5.68 d J=3.3	H-14[e] H-44[b]
18	172.8		H-16[b] H-19a[b] H-19b[e]
19	40.1	a 3.95 d J=17.2	
		b 2.85 m	
20	57.8	4.47 m	
21	68.4	4.48 m	H-53[c]
22		7.50 d J=9.3	
23	172.1		H-25[e] H-56[b]
24	58.5	4.89 dd J=5.9, 2.3	
25	71.1	5.90 dq J=6.2, 2,3	
28	34.3	3.32 m	H-2[e] H-30[b]
29	130.4		H-28[b]
30	130.7	6.78 d J=8.7	H-28[b]
31	114.2	6.69 d J=8.7	H-30[b,d]
32	159.0		H-35[b,d] H-30[b]
35	54.9	3.34 s	H-31[b]
36	38.4	2.15 s	
39	42.1	1.70 m	
40	25.3	a 1.80 m	H-41[b]
		b 1.48 m	
41	23.8	0.94 d J=6.3	H-42[c]
42	21.4	0.98 d J=6.3	
44	15.7	1.78 d J=6.9	
46	30.9	2.51 dt J=6.8, 3.3	H-16[d]
47	17.1	0.89 d J=6.8	H-48[c]
48	23.5	0.85 d J=6.8	H-47[c]
50		2.82 m	
51	27.9	2.45 dt J=6.7, 3.3	
52	17.0	1.06 d J=6.7	
53	21.0	1.19 d J=6.7	
54			
56		8.03 J=5.9	
57	172.4		H-58[d,e]
58	55.2	5.64 dd J=10.5, 3.3	H-56[c]
60	173.0		H-74[b,c,d,e]
61	57.4	4.33 dd J=8.2, 4.8	
62	28.2	a 1.47 m	
		b 1.39 m	
63	25.7	a 1.51 m	
		b 1.11 m	
64	46.7	a 2.89 t J=10.0	
		b 2.79 t J=10.0	
66	174.2		H-68[b]
67	66.3	4.12 m	
68	20.4	1.28 d J=6.7	
70	36.5	a 2.05 dt J=10.5, 4.0	
		b 1.61 dt J=10.5, 4.0	
71	25.2	1.34 m	
72	21.5	0.91 d J=6.7	
73	19.0	0.85 d J=6.7	
74	31.1	2.81 s	
77		3.72 d J=10.0	
78	17.2	1.84 d J=6.2	

[a]Varian VXR-500 NMR spectrometer
[b]Long range HETCOR J_{CH}=10 Hz Varian XL-400
[c]Long range HETCOR J_{CH}=6 Hz Varian XL-400
[d]COLOC J_{CH}=6Hz IBM AF-200
[e]INAPT Varian XL-400

^{13}C[a]	^{1}H[a] mult J=Hz	Didemnin B Long Range ^{1}H-^{13}C correl.
169.1		H-2[b]
66.1	3.17 dd J=10.4, 5.5	
170.5		H-36[b]
56.3	4.15 m	H-36[b]
28.1	a 1.48 m	H-7b[b]
	b 1.36 m	
24.5	a 1.43 m	
	b 1.11 m	
46.6	a 2.85 dt J=7.1, 2.9	
	b 2.68 dt J=7.1, 2.9	
171.7		H-11[b] H-12[b]
50.0	5.12 brt J=10.2	
	8.20 d J=9.4	
169.9		H-14[b] H-44[b]
50.1	4.69 q J=6.7	
204.4		H-14[b] H-44[b]
81.3	5.68 d J=3.4	
172.8		H-16[b] H-19b[b]
40.2	a 4.12 d J=9.4	
	b 2.85 m	
66.3	4.48 brdt J=4.3	
68.1	4.62 dt J=9.9, 2.8	H-19b[b] H-20[b]
	7.42 d J=15.3	
169.4		H-22[b]
58.5	4.89 dd J=5.9, 2.0	H-78[b]
71.1	5.92 dq J=6.2, 2.0	
34.4	3.32 dt J=13.0, 5.5	
130.6		H-30[b]
130.7	6.67 d J=8.7	H-28[b] H-31[b]
114.0	6.65 d J=8.7	
159.0		H-31[b] H-35[b]
54.9	3.35 s	
38.3	2.15 s	
42.1	1.74 m	H-42[b]
25.6	a 1.80 m	
	b 1.48 m	
23.8	1.01 d J=5.8	
21.4	1.05 d J=5.8	H-39[b]
15.7	1.78 d J=6.7	H-14[b]
31.0	2.49 dq J=6.9, 3.4	
17.0	0.90 d J=6.9	H-48[b]
23.5	0.85 d J=6.9	H-47[b]
	2.82 m	
34.4	2.30 dq J=6.8, 2.8	H-54[b]
27.4	1.78, 1.48 m	
14.9	1.16 t J=7.3	
25.3	1.08 d J=6.8	
	8.02 d J=5.9	
172.2		H-56[b] H-58[b]
55.2	5.63 dd J=11.6, 3.9	
173.0		H-74[b] H-56[b]
57.4	4.35 dd J=8.3, 4.6	
28.2	a 1.48 m	
	b 1.23 m	
25.3	a 1.45 m	
	b 1.33 m	
46.6	a 3.38 t J=9.6	
	b 3.03 t J=9.6	
174.3		H-68[b]
58.5	4.12 m	
20.4	1.28 d J=6.6	
36.6	a 2.07 dt J=10.4, 3.9	
	b 1.64 dt J=10.4, 3.9	
25.6	1.35 m	
21.5	0.98 d J=6.7	H-73[b]
19.0	0.85 d J=6.7	H-72[b]
31.1	2.88 s	H-61[b]
	3.72 d J=10.0	
17.0	1.92 d J=6.2	

Table 1

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10. The peptides were isolated from the lyophilized, ground tunicate by size exclusion (Sephadex LH-20) chromatography of the methanol soluble material followed by flash silica gel chromatography. Final purification was achieved by C18 RP HPLC (80:20 MeOH/H_2O) to yield didemnin B (688 mg; 0.13%) and nordidemnin B (324 mg; 0.06%). Cytotoxic towards L 1210 murine leukemia cells (ID_{50} 7.5 x 10^{-4} and 2.7 x 10^{-3} µg/ml for didemnin B and nordidemnin B, respectively. Also see reference 7.

11. Identification made at collection based on morphological and visual characteristics.

12. Rinehart had suggested that lower homologues of the didemnins were present but had not isolated them. See ref. 3

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