Chondropsin D, a New 37-Membered-Ring Macrolide Lactam from the Marine Sponge *Chondropsis* Species

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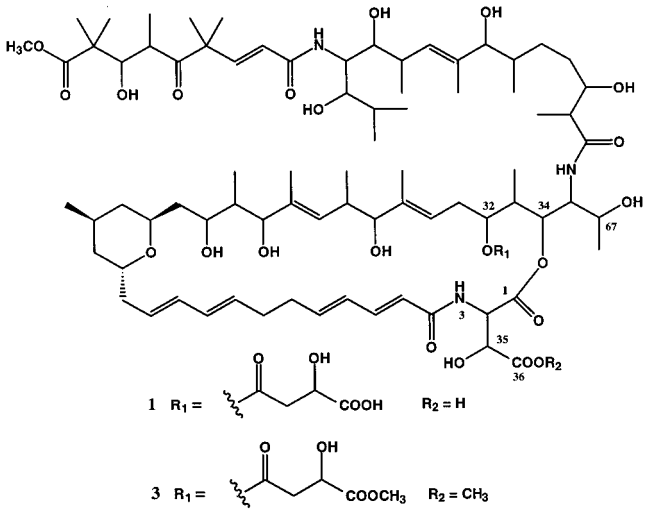
Chondropsin D (**2**), a new 37-membered-ring macrolide lactam, was isolated as a minor constituent of an aqueous extract of the marine sponge *Chondropsis* sp. This sponge sample had previously been the source of chondropsins A (**1**) and B, two novel polyketide-derived macrolides with potent cytotoxic activity. The structure of **2** was initially deduced from analysis of spectral data. This assignment was supported by the observation that chondropsin A (**1**), which contains a 35-membered macrocyclic ring, could be converted to chondropsin D (**2**) by a base-catalyzed intramolecular transesterification reaction. Rearrangement of the methylated derivative of chondropsin A (**3**) to the corresponding methylated analogue of chondropsin D (**4**) confirmed the structure of **2**.

We recently described a novel family of highly functionalized macrolide lactams, which we obtained from several different marine sponge sources. Chondropsins A (**1**) and B were isolated from an Australian collection of *Chondropsis* sp.,[1] while a sample of *Ircinia ramosa* from Australia provided 73-deoxychondropsin A,[2] and an *Ircinia* sp. collected in the Philippines yielded chondropsin C.[2] The chondropsin macrolides comprise a unique class of secondary metabolites in which a series of structural elements of apparent polyketide biosynthetic origin are coupled via amide linkages. They each contain a complex acyclic fragment and a 35-membered macrocycle which incorporates both amide and ester linkages to effect ring closure. The chondropsins exhibited potent and differential antiproliferative and cytotoxic properties in the National Cancer Institute (NCI)'s 60-cell antitumor screen.[3,4] They provided mean-panel GI_{50} values of approximately (2.4−3.6) × 10^{-8} M, and Compare-algorithm analyses of their mean-graph profiles[4] showed no significant correlations with any mean-graph profiles in the NCI's standard agents database. It appears that the mechanism of tumor cell inhibition by the chondropsins is different from that of conventional antitumor agents, and thus they represent an interesting new antiproliferative structural lead class.

The aqueous extract (44 g) of the Australian sponge *Chondropsis* sp. was selected for detailed chemical study based on the NCI's 60-cell screening results. Cytotoxicity-guided fractionation of the extract on reversed-phase C_4 media, Sephadex LH-20, and C_{18} HPLC provided chondropsins A (**1**) and B as the principal cytotoxic constituents.[1] Another minor compound was recovered during the HPLC purification step, and by ^{1}H NMR it appeared to be closely related to chondropsins A (**1**) and B. Repeated C_{18} HPLC eluted with a linear CH_3CN-H_2O gradient ultimately provided a total of 3.5 mg of the purified minor compound, which was given the name chondropsin D (**2**).



The molecular formula of chondropsin D (**2**) was established by HRFABMS to be $C_{83}H_{133}N_3O_{26}$, which indicated that it was isomeric with chondropsin A (**1**). The IR and UV spectra recorded for **2** were also virtually identical to those obtained with **1**. A comprehensive set of 1-D and 2-D NMR data for **2** were collected and analyzed. It was clear that most of the structural features found in **1** were also present in **2**, and it was possible to make a complete assignment of the ^{1}H and ^{13}C resonances (Table 1). The only apparent structural difference between **1** and **2** was the position of the ester link in the macrocycle. In chondropsin A (**1**) an ester bridge was formed between the C-1 carbonyl and the oxygen on C-34. The H-34 resonance in chondropsin D (**2**)[5] was shifted upfield to δ 3.66 (versus δ 5.11 in **1**), which suggested that C-34 was now substituted with a hydroxyl group. In addition, H-67 in **2** was deshielded to δ 5.03 (versus δ 3.78 in **1**), which was consistent with an ester linkage at this position. While the location of the ester was firmly established in **1** by an HMBC correlation from H-34 to C-1, no correlation was observed in **2** between H-67 and C-1, even when HMBC data were recorded under a variety of different experimental conditions. A fortuitous observation revealed that when chondropsin A (**1**) was dissolved in pyridine-d_5, it slowly rearranged to a new

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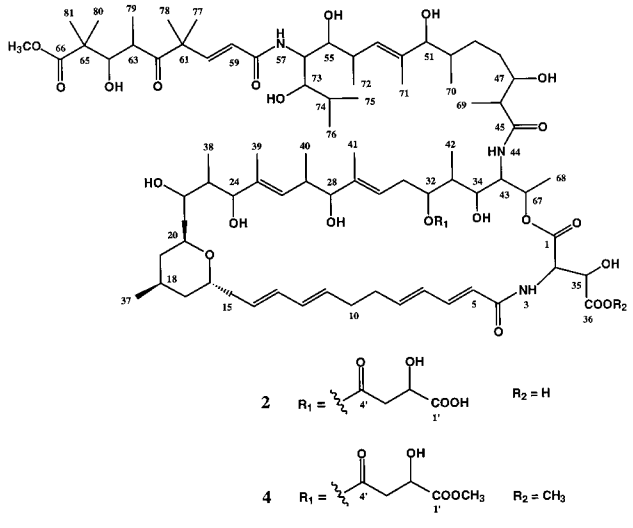
Table 1. ^{1}H and ^{13}C NMR Data for Chondropsin D (**2**) in DMF-d_7

pos.	δ_C mult[a]	δ_H mult (*J* in Hz)	HMBC	pos.	δ_C mult[a]	δ_H mult (*J* in Hz)	HMBC
1	172.6 s			44			7.43 d (9.0)
2	55.9 d	5.13 dd (9.0, 2.2)	C-35	45	176.6 s		
3		8.16 d (9.0)		46	47.2 d	2.47 m	
4	167.6 s[b]			47	73.6 d	3.52 m	
5	124.4 d	6.29 d (15.0)	C-4[b]	48	33.2 t	1.48 m	
6	140.4 d	7.11 dd (15.0, 11.0)	C-4[b], C-7	49	30.1 t	1.17 m, 1.31 m	
7	129.7d	6.26 dd (15.0, 11.0)		50	36.4 t	1.55 m	
8	141.6 d	6.10 m		51	83.1 d	3.52 d (8.0)	
9	34.0 t[c]	2.25 m		52	137.3 s		
10	32.3 t	2.18 m	C-12	53	129.6 d	5.49 d (10.0)	C-51, C-55, C-71
11	131.5 d	5.59 dt (15.0, 7.5)		54	35.3 d	2.67 m	
12	132.0 d	6.12 m	C-10	55	74.5 d	3.76 m	
13	132.1 d	6.11 m	C-15	56	53.7 d[e]	4.05 m	C-55, C-58
14	130.8 d	5.72 dt (15.0, 8.0)	C-12, C-13	57		7.59 d (10.0)	C-58
15	34.4 t[c]	2.05 m, 2.72 m		58	165.9 s		
16	73.2 d	3.87 m		59	124.7 d	6.36 d (15.5)	C-58, C-61
17	37.9 t	Hβ 1.25 m, Hα 1.49 m		60	146.7 d	6.88 d (15.5)	C-58, C-59, C-62, C-77
18	25.6 d	1.81 m		61	51.3 s		
19	41.7 t[d]	Hβ 0.80 m, Hα 1.50 m		62	214.7 s		
20	66.7 d	3.72 m		63	44.4 d	3.20 dq (9.5, 6.5)	C-62, C-64, C-79
21	42.8 t	1.44 m, 1.49 m		64	77.2 d	4.03 dd (9.5, 2.5)	
22	67.7 d	4.15 bd (10.5)		65	46.7 s		
23	41.9 d[d]	1.49 m		66	178.0 s		
24	80.2 d	3.91 d (9.5)	C-22, C-23, C-26, C-39	67	74.4 d	5.00 m	
25	137.9 s			68	17.6 q	1.23 d (6.0)	C-43, C-67
26	130.7 d	5.27 m	C-24, C-28, C-39	69	15.3 q	1.12 d (7.0)	C-45, C-46, C-47
27	36.5 d	2.61 m		70	15.9 q	0.95 d (7.0)	C-49, C-50, C-51
28	81.6 d	3.71 d (9.0)	C-27, C-29, C-30, C-40	71	11.7 q	1.47 s	C-51, C-52, C-53
29	140.0 s			72	18.1 q	1.00 d (6.5)	C-53, C-54, C-55
30	121.5 d	5.30 m	C-28, C-32, C-41	73	75.6 d	3.60 m	
31	30.1 t	2.22 m, 2.52 m		74	31.5 d	1.48 m	
32	74.3 d	5.23 bt (7.0)	C-1′	75	19.6 q	0.87 d (7.0)	C-73, C-74, C-76
33	38.8 d	1.82 m		76	20.1 q	0.94 d (7.0)	C-73, C-74, C-75
34	72.6 d	3.50 m		77	23.8 q	1.20 s	C-60, C-61, C-62
35	72.0 d	4.73 d (2.0)		78	23.7 q	1.27 s	C-60, C-61, C-62
36	171.6 s[b]			79	15.3 q	0.76 d (6.5)	C-62, C-63, C-64
37	22.8 q	0.86 d (6.5)	C-17, C-18, C-19	80	17.7 q	1.11 s	C-64, C-65, C-81
38	10.9 q	0.71 d (7.0)	C-22, C-23, C-24	81	25.3 q	1.17 s	C-64, C-65, C-80
39	12.6 q	1.62 s	C-24, C-25, C-26	1′	174.1 s		
40	18.2 q	0.84 d (6.5)	C-26, C-27, C-28	2′	68.8 d	4.52 dd (8.4, 4.0)	C-4′
41	12.0 q	1.59 s	C-28, C-29, C-30	2′	40.3 t	2.67 m, 2.73 m	C-1′, C-4′
42	10.1 q	0.92 d (7.0)	C-32, C-33, C-34	4′	173.5 s		
43	53.6 d[e]	4.18 m		OCH_3	51.7 q	3.62 s	C-66

[a] Multiplicity inferred from a DEPT pulse sequence. [b] Assignments based on HMBC correlations seen in **4**. [c,d,e] Assignments may be interchanged.

compound. After HPLC purification and detailed NMR analysis, it was evident that the newly formed compound was identical to chondropsin D (**2**). Thus, compound **2** can arise from **1** via a transesterification reaction in which the ester migrates from O-34 to O-67. While the base-catalyzed conversion of **1** to **2** confirmed that these two compounds shared the same structural features, the question still remained of which carbonyl group (C-1 vs C-36) was involved in the ester link in **2** and which one existed as a free carboxylic acid. NOESY and 1-D NOE experiments with **2** failed to provide proof of the ester orientation. In chondropsin A (**1**), the free carboxylic acid at C-36 had been established by preparation of the methylated derivative **3** and measurement of NOE interactions between H-35 and the C-36 OMe group.[1] A similar approach was taken with **2**, so it was treated with CH_2N_2 to give the methylated derivative **4**. However, NOE studies with **4** failed to unambiguously define where the OMe group was located. Fortunately, compound **3** could also be induced to undergo a base-catalyzed transesterification analogous to that observed with **1**. Thus, a solution of **3** in pyridine-d_5 slowly rearranged to give a new compound that was identical to the methylated derivative **4**. This confirmed that the macrocyclic ester link incorporated the C-1 carbonyl and that chondropsin D (**2**) has the structure as illustrated.



Since chondropsin D (**2**) can arise from a rearrangement of chondropsin A (**1**), it is possible that **2** is an artifact that resulted during handling of the extract. However, careful monitoring of various solutions of chondropsin A (**1**) in CH_3OH, CH_3CN, DMSO, CH_3OH-H_2O, and CH_3CN-H_2O did not reveal any conversion to **2**. In addition, we could

Table 2. ^{1}H and ^{13}C[a] NMR Data for Methyl Ester Derivative **4** in DMF-d_7

pos.	δ_C	δ_H mult (J in Hz)	pos.	δ_C	δ_H mult (J in Hz)	pos.	δ_C	δ_H mult (J in Hz)
2	55.7	5.15 dd (9.0, 2.0)	30	121.2	5.30 m	59	124.5	6.36 d (15.5)
3		8.30 d (9.0)	31	30.8	2.22 m, 2.45 m	60	146.6	6.87 d (15.5)
5	124.7	6.29 d (15.0)	32	74.9	5.24 bt (6.5)	63	44.5	3.20 dq (10.0, 7.0)
6	140.7	7.15 dd (15.0, 11.0)	33	39.5	1.87 m	64	77.2	4.04 m
7	129.5	6.23 dd (16.0, 11.0)	34	72.3	3.50 m	67	73.8	5.00 pent (6.0)
8	141.7	6.07 m	35	72.0	4.71 d (2.0)	68	17.9	1.23 d (6.0)
9	33.0	2.27 m	37	22.7	0.86 d (7.0)	69	15.2	1.11 d (7.0)
10	32.3	2.19 m	38	10.9	0.72 d (7.0)	70	16.0	0.95 d (6.5)
11	131.4	5.60 m	39	12.6	1.63 s	71	11.7	1.48 s
12	132.3	6.10 d (14.9)	40	18.4	0.88 d (6.5)	72	18.1	0.99 d (7.0)
13	132.3	6.10 d (14.9)	41	12.0	1.59 s	73	75.7	3.60 m
14	130.9	5.70 m	42	10.1	0.91 d (7.0)	74	31.5	1.48 m
15	34.5	2.04 m, 2.68 m	43	53.4	4.17 m	75	19.5	0.87 d (7.0)
16	73.4	3.85 m	44		7.40 d (9.5)	76	20.1	0.93 d (7.0)
17	37.8	Hβ 1.27 m, Hα 1.50 m	46	47.2	2.51 m	77	23.8	1.20 s
18	25.5	1.80 m	47	73.9	3.52 m	78	23.7	1.26 s
19	41.7	Hβ 0.80 m, Hα 1.58 m	48	33.0	1.46 m	79	15.3	0.76 d (7.0)
20	66.5	3.77 m	49	29.9	1.28 m	80	17.7	1.10 s
21	42.8	1.20 m, 1.46 m	50	36.2	1.55 m	81	25.2	1.17 s
22	67.3	4.20 m	51	83.1	3.54 m	2′	68.8	4.52 dd (7.5, 5.0)
23	41.5	1.50 m	53	130.4	5.49 d (10.0)	3′	40.2	2.78 m, 2.82 m
24	80.2	3.91 d (9.0)	54	35.3	2.67 m	36-OCH_3	52.6	3.73 s
26	130.0	5.30 m	55	74.6	3.76 m	66-OCH_3	51.7	3.62 s
27	36.7	2.61 m	56	53.6	4.07 m	1′-OCH_3	51.8	3.66 s
28	81.1	3.74 m	57		7.57 d (10.5)			

[a] Only protonated carbons could be assigned from an HSQC experiment.

not detect the conversion of **1** to **2** on Sephadex LH-20 or reversed-phase chromatographic media. The only condition found to induce the rearrangement of **1** was prolonged exposure to pyridine. Direct LC-MS analysis of the crude *Chondropsis* sp. extract verified the presence of chondropsin D (**2**) in approximately the same ratio to the other chondropsins that we observed in our large-scale workup of the extract. While chondropsin D (**2**) does not appear to be an artifact of the isolation and purification procedures that we utilized, lack of access to fresh sponge material precluded an assessment of its presence in the source organism. A purified sample of chondropsin D (**2**) dissolved in pyridine for 5 days gradually generated a small amount of chondropsin A (**1**), as evidenced by HPLC analysis. Thus, the transesterification reaction between **1** and **2** is an equilibrium process, although the rate of conversion of **1** to **2** is considerably faster than the reverse reaction.

Chondropsin D (**2**) was evaluated for cytotoxic activity toward melanoma (LOX) and leukemia (MOLT-4) human tumor cell lines in a 2-day in vitro assay, details of which have been described previously.[6] Compound **2** exhibited IC_{50}'s of approximately 10 and 250 ng/mL toward the LOX and MOLT-4 cell lines, respectively.

Experimental Section

General Experimental Procedures. NMR spectra were acquired in DMF-d_7 and pyridine-d_5 on a Varian Unity INOVA spectrometer operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C and referenced to the residual nondeuterated solvent. FAB mass spectra were recorded on a JEOL SX102 spectrometer using glycerol or nitrobenzyl alcohol as matrix. Electrospray mass spectra were acquired on a Hewlett-Packard HP1100 integrated LC-MS system. IR spectra were obtained from neat samples on KCl disks in a Perkin-Elmer Spectrum 2000 FT-IR spectrometer. Optical rotations were measured on a Perkin-Elmer 241 polarimeter, and UV spectra were recorded on a Beckman DU 640 spectrophotometer.

Animal Material. Samples of *Chondropsis* sp. (order Poecilosclerida, family Desmacidonidae) were collected in 1988 at a depth of approximately 20 m along the shore of Bass Island, Wollongong, Australia. Samples were kept frozen prior to extraction. Taxonomic identification was made by Christopher Battershill, in association with the Australian Institute of Marine Science, and a voucher specimen (#Q66C1004) has been deposited with the Smithsonian Institution, Washington, D.C.

Extraction and Isolation. The frozen sponge (357 g, wet weight) was ground in dry ice and then extracted with H_2O at 4 °C. The aqueous extract was removed by centrifugation and subsequently lyophilized to give 44.0 g of crude extract. The general fractionation and isolation procedures employed with this extract have previously been described.[1] Final purification of chondropsin D (**2**) was achieved by C_{18} reversed-phase HPLC (Dynamax ODS, 10 × 250 mm, 8 μm; flow rate, 3 mL/min) using a linear CH_3CN-H_2O gradient (45:55 to 100:0 over 30 min). A total of 3.5 mg (0.001% wet weight) of **2** was ultimately obtained.

Chondropsin D (2): white gum, $[\alpha]^{27}_D$ −5.0° (*c* 0.06, MeOH); UV [MeOH] λ_{max} (log ϵ) 225 (4.18), 262 (4.01) nm; IR ν_{max} (KCl) 3500−3300,1680, 1610, 1532, 1200, 1180 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; FABMS $(M + Na)^+$ *m/z* 1610.9; HRFABMS CsI doped sample, $(M - H + 2Cs)^+$ *m/z* 1852.7283, calcd for $C_{83}H_{132}N_3O_{26}Cs_2$, 1852.7201.

Methylation of 2. A solution of 0.2 mg of chondropsin D (**2**) in 1.0 mL of MeOH was treated at room temperature with an excess of CH_2N_2 in diethyl ether (3.0 mL). The solvent was removed under a stream of N_2, and the residue was dissolved in MeOH and purified by C_{18} HPLC (eluted with a linear gradient from 45 to 100% CH_3CN in H_2O containing 0.1% TFA) to give 0.1 mg of the methylated derivative **4**: ^{1}H NMR data see Table 2; FABMS $(M + Na)^+$ *m/z* 1638.9, appropriate for $C_{85}H_{137}N_3O_{26}Na$.

Conversion of Chondropsin A (1) to Chondropsin D (2). A 2.0 mg solution of chondropsin A (**1**) in pyridine-d_5 was kept at room temperature, and ^{1}H NMR spectra were periodically acquired. Within 1 day new ^{1}H resonances were observed, and these signals gradually increased over time. After 7 days, the pyridine was removed under reduced pressure and the residue was purified by HPLC as described above to give 0.6 mg of a compound that by FABMS, HPLC retention time, ^{1}H NMR, and HSQC data was identical to chondropsin D (**2**).

Conversion of the Methylated Derivative 3 to 4. A 2.0 mg solution of **3** in pyridine-d_5 was treated in a manner identical to that described above to give 0.8 mg of a compound that by FABMS, HPLC retention time, co-injection, and ^{1}H NMR data was identical to **4**.

Antiproliferative Bioassay. A DMSO solution of chondropsin D (**2**) was assayed for antiproliferative properties using LOX (melanoma) and MOLT-4 (leukemia) human tumor cell lines. Experimental details of this 2-day, in vitro assay have been described previously.[6]

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(5) To facilitate spectral comparisons, the numbering scheme used for chondropsin D (**2**) is the same as that originally used for chondropsin A (**1**).[1]
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