Pergamon

Tetrahedron Letters 42 (2001) 1623–1626

TETRAHEDRON LETTERS

New chondropsin macrolide lactams from marine sponges in the genus *Ircinia*

Mohammad A. Rashid,[a] Kirk R. Gustafson[b] and Michael R. Boyd[b,*]

[a]*SAIC-Frederick, Frederick, MD 21702-1201, USA*
[b]*Laboratory of Drug Discovery Research and Development, Division of Basic Sciences, NCI-Frederick, Building 1052, Room 121, Frederick, MD 21702-1201, USA*

Received 3 November 2000; revised 6 December 2000; accepted 7 December 2000

Abstract—Two new polyketide-derived macrolide lactams, identified as 73-deoxychondropsin A (**2**) and chondropsin C (**3**), have been isolated from two different collections of marine sponges which belong to the genus *Ircinia*. An Australian collection of *Ircinia ramosa* provided 73-deoxychondropsin A (**2**), while samples of *Ircinia* sp. collected in the Philippines yielded chondropsin C (**3**). The structures of **2** and **3** were assigned by interpretation of their spectral data. © 2001 Published by Elsevier Science Ltd.

We recently described the isolation of chondropsins A (**1**) and B from the sponge *Chondropsis* sp.[1] These compounds represent a novel class of complex polyketide macrolides with potent in vitro antiproliferative and cytotoxic activities. The chondropsins produced a distinct pattern of differential growth inhibition in the NCI's 60-cell antitumor screen,[2] and COMPARE-algorithm analyses of their mean-graph profiles[3] with the NCI natural product repository extracts database identified two different *Ircinia* sponge extracts with mean-graph profiles similar to the chondropsins. We initiated cytotoxicity-guided fractionation of these *Ircinia* extracts to identify the active constituents. A series of cytotoxic macrolides have recently been described from an Okinawan *Ircinia* sp.[4] however, these metabolites are not structurally related to the chondropsins.

The aqueous extract (27.5 g) of *Ircinia ramosa* collected in Australia was fractionated on C_4 reversed-phase media, Sephadex LH-20, and C_{18} HPLC (eluted with a 45–100% gradient of CH_3CN in H_2O with 0.1% TFA) to give chondropsin A (**1**)[1] (1 mg) and a new compound identified as 73-deoxychondropsin A (**2**)[†] (5 mg). HRFABMS established the molecular formula of **2** as $C_{83}H_{133}N_3O_{25}$, which only differed from **1** by a lack of one oxygen atom. The ^1H and ^{13}C NMR spectra of **2** (Table 1) were virtually superimposable with those of **1**. The only significant spectral differences between the two compounds occurred in a region centered around C-73. It was apparent that the oxymethine at C-73 in **1** was replaced with a methylene in **2**. HMBC correlations observed from H-53 (δ 5.42) and NH-57 (δ 7.62) to C-55 (δ 78.2) confirmed the presence of a C-55 oxymethine group in 2, while an HMBC correlation from H-55 (δ 3.36) to C-73 (δ 40.3) established the position of the new methylene group. A DEPT experiment confirmed that the carbon at δ 40.3 had two attached protons and COSY correlations from H-56 (δ 4.07) to the heavily overlapped region of the H_2-73 protons (δ 1.46 and 1.54) were consistent with the presence of a methylene at C-73. Treatment of **2** with diazomethane provided a bis methyl ester derivative (MNa^+, m/z 1622.9) and data from a comprehensive set of 2-D NMR experiments with **2** verified that the only difference between **1** and **2** was at C-73. Thus, the new compound was assigned to be 73-deoxychondropsin A (**2**).

Keywords: macrolide lactams; polyketide; chondropsins; *Ircinia ramosa*.

* Corresponding author. Tel.: 301-846-5391; fax: 301-846-6919; e-mail: boyd@dtpax2.ncifcrf.gov

† Compound **2**: white powder; $[\alpha]_D$ +2.0 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 216 (4.62), 226 (4.61), 261 (4.56) nm; IR υ_{max} (film) 3500–3200, 1660, 1620, 1532, 1204, 1138, 998 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HRFABMS (CsI-doped) obs. $[M+Cs]^+$, m/z 1704.8308, $C_{83}H_{133}CsN_3O_{25}$ requires 1704.8279.

0040-4039/01/$ - see front matter © 2001 Published by Elsevier Science Ltd.
PII: S0040-4039(00)02348-0

Table 1. ^{1}H and ^{13}C NMR data for 73–deoxychondropsin A (**2**) in DMF-d_7[a]

Pos.	δ_C Mult.[b]	δ_H Mult. (J in Hz)	Pos.	δ_C Mult.[b]	δ_H Mult. (J in Hz)	Pos.	δ_C Mult.[b]	δ_H Mult. (J in Hz)
1	171.9 s		30	123.4 d	5.22 t (6.5)	59	124.5 d	6.31 d (15.5)
2	55.5 d	5.15 m	31	31.8 t	2.05 m, 2.45 m	60	146.8 d	6.89 d (15.5)
3		7.91 d (9.5)	32	73.1 d	4.85 m	61	51.3 s	
4	167.5 s		33	38.6 d	2.00 m	62	214.7 s	
5	124.4 d	6.30 d (15.0)	34	77.0 d	5.10 m	63	44.6 d	3.20 dq (10.0, 7.0)
6	140.7 d	7.14 dd (15.0, 10.5)	35	72.1 d	4.82 bs	64	77.2 d	4.04 d (10.0)
7	129.8 d	6.28 dd (15.0, 10.5)	36	171.8 s		65	46.7 s	
8	142.1 d	6.12 m	37	22.8 q	0.87 d (6.6)	66	178.0 s	
9	34.5 t	2.29 m	38	9.8 q	0.62 d (7.0)	67	69.3 d	3.78 m
10	33.0 t	2.15 m	39	11.2 q	1.57 s	68	21.1 q	1.08 d (6.2)
11	131.3 d	5.70 m	40	18.1 q	0.65 d (6.0)	69	15.5 q	1.13 d (7.0)
12	131.9 d	6.17 d (14.9)	41	11.3 q	1.55 s	70	15.8 q	0.93 d (7.0)
13	132.0 d	6.17 d (14.9)	42	9.7 q	1.03 d (7.0)	71	12.2 q	1.54 s
14	132.0 d	5.67 m	43	53.3 d	4.15 m	72	17.8 q	0.96 d (7.0)
15	34.6 t	2.03 m, 2.79 m	44		7.50 d (10.0)	73	40.3 t	1.46 m, 1.54 m
16	72.3 d	4.00 m	45	176.7 s		74	25.2 d	1.56 m
17	37.9 t	Hβ 1.27 m, Hα 1.48 m	46	47.3 d	2.54 m	75	24.4 q	0.86 d (6.0)
18	25.9 d	1.85 m	47	73.5 d	3.52 m	76	21.8 q	0.89 d (6.0)
19	41.7 t	Hβ 0.82 m, Hα 1.52 m	48	33.1 t	1.48 m	77	23.8 q	1.20 s
20	65.9 d	3.69 m	49	29.3 t	1.21 m, 1.47 m	78	23.9 q	1.26 s
21	42.9 t	1.24 m, 1.46 m	50	36.2 t	1.57 m	79	15.3 q	0.76 d (7.0)
22	66.0 d	4.25 m	51	83.1 d	3.57 m	80	17.7 q	1.10 s
23	41.8 d	1.45 m	52	137.2 s		81	25.2 q	1.17 s
24	80.2 d	3.86 d (9.0)	53	130.4 d	5.42 d (9.5)	1′	172.6 s	
25	138.0 s		54	35.7 d	2.64 m	2′	68.8 d	4.52 dd (8.4, 4.0)
26	132.1 d	5.12 m	55	78.2 d	3.36 m	3′	40.2 t	2.63 m, 2.78 m
27	36.4 d	2.50 m	56	50.4 d	4.07 m	4′	172.9 s	
28	82.4 d	3.51 d (8.1)	57		7.62 d (10.0)	OCH_3	51.7 q	3.62 s
29	138.7 s		58	165.4 s				

[a] ^{1}H and ^{13}C spectra acquired at 500 and 125 MHz, respectively.
[b] Multiplicity inferred from the DEPT pulse sequence.

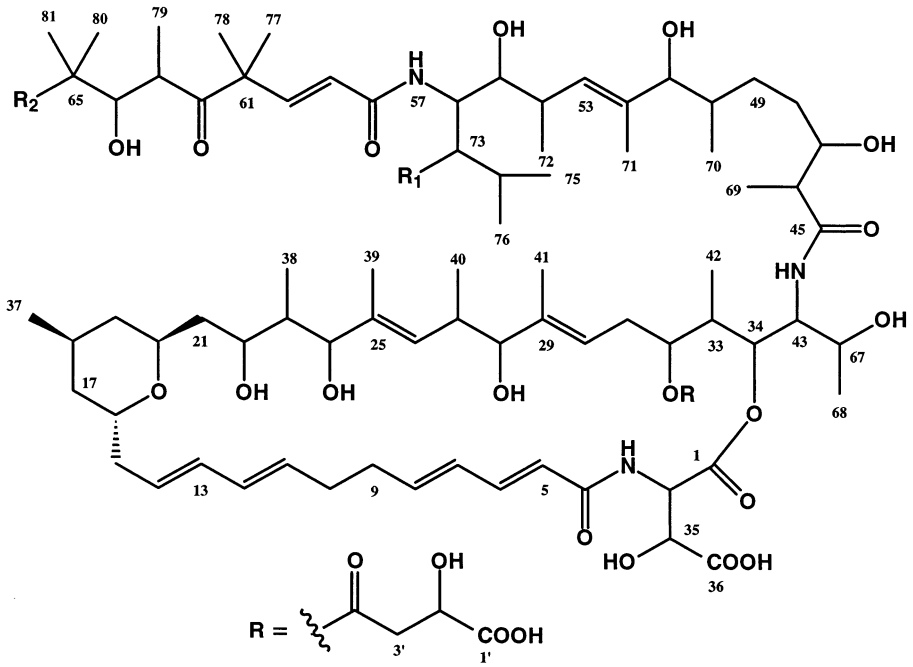

Table 2. ^{1}H and ^{13}C NMR data for chondropsin C (**3**) in CD_3OH[a]

Pos.[b]	δ_C Mult.[c]	δ_H Mult. (J in Hz)	HMBC	Pos.[b]	δ_C Mult.[c]	δ_H Mult. (J in Hz)	HMBC
1	172.7 s			43	54.1 d	4.11 m	C-45, C-67
2	56.0 d	5.15 m	C-1, C-4, C-35, C-36	44		7.45 d (10.0)	C-45
3		7.73 d (8.5)	C-2, C-4	45	178.9 s		
4	169.8 s			46	48.5 d	2.50 m	C-45, C-47, C-69
5	124.0 d	6.22 d (15.0)	C-4, C-7	47	74.3 d	3.52 m	
6	142.5 d	7.13 dd (15.0, 11.0)	C-4	48	33.2 t	1.50 m, 1.54 m	
7	130.1 d	6.27 dd (15.0, 11.0)	C-8, C-9	49	29.7 t	1.18 m, 1.30 m	C-50, C-47
8	143.6 d	6.15 m	C-6, C-9, C-10	50	36.8 t	1.61 m	
9	35.6 t	2.30 m	C-8, C-10	51	84.1 d	3.66 d (8.0)	C-49, C-52, C-70, C-71
10	33.6 t	2.13 m, 2.19 m		52	137.7 s		
11	131.9 d	5.70 m		53	131.2 d	5.36 d (10.0)	
12	132.4 d	6.17 bd (15.0)	C-10, C-14	54	36.2 d	2.66 m	
13	132.6 d	6.14 bd (15.0)	C-14, C-15	55	78.9 d	3.36 dd (11.0, 5.5)	C-53, C-56, C-72, C-73
14	132.9 d	5.67 m	C-13, C-16	56	51.4 d	4.03 m	
15	35.2 t	2.06 m, 2.78 m		57		7.80 d (10.2)	C-56, C-58
16	73.0 d	4.06 m	C-20[d]	58	167.6 s		
17	38.2 t	Hβ 1.31 m, Hα 1.52 m	C-15, C-16	59	124.1 d	6.10 d (16.0)	C-58, C-61
18	26.6 d	1.87 m		60	148.6 d	6.93 d (16.0)	C-58, C-59, C-62, C-77
19	41.8 t	Hβ 0.86 m, Hα 1.53 m		61	52.0		
20	66.7 d	3.68 m		62	217.2 s		
21	42.7 t	1.23 m, 1.50 m	C-19, C-20	63	45.8 d	3.16 dq (9.5, 6.5)	C-62, C-64, C-65
22	66.9 d	4.22 bd (10.5)	C-21, C-24, C-38	64	78.7 d	3.56 dd (9.5, 2.5)	C-63, C-80, C-81
23	41.9 d	1.55 m		65	30.1 d	1.27 m	
24	81.4 d	3.81 d (9.5)	C-22, C-26, C-39	67	70.2 d	3.77 m	
25	137.9 s			68	21.8 q	1.10 d (6.5)	C-43, C-67
26	134.6 d	5.02 m	C-24, C-27, C-40	69	15.7 q	1.14 d (6.5)	C-45, C-46, C-47
27	36.3 d	2.47 m		70	15.9 q	0.96 d (6.5)	C-49, C-50, C-51
28	84.1 d	3.43 d (9.0)		71	12.0 q	1.53 s	C-51, C-52, C-53
29	138.5 s			72	17.9 q	0.98 d (6.5)	C-53, C-54, C-55
30	124.1 d	5.20 t (6.5)	C-28, C-41	73	40.3 t	1.45 m, 1.48	
31	32.7 t	2.05 m, 2.45 m	C-30, C-32	74	25.8 d	1.56 m	
32	73.7 d	4.84 m	C-4′[d]	75	24.4 q	0.91 d (7.0)	C-73, C-74, C-76
33	39.1	1.93 m	C-34, C-42	76	22.0 q	0.90 d (7.0)	
34	78.2 d	5.06 m	C-1, C-33, C-67	77	23.8 q[e]	1.25 s	C-60, C-61, C-62
35	72.4 d	4.85 m		78	23.9 q[e]	1.28 s	C-60, C-61, C-62
36	172.7 s			79	15.7 q	0.87 d (6.5)	C-62, C-64
37	22.8 q	0.89 d (6.5)	C-17, C-18, C-19	80	14.3 q	0.82 d (6.5)	C-64, C-65, C-81
38	9.3 q	0.58 d (7.0)	C-22, C-23, C-24	81	20.5 q	0.94 d (7.0)	C-64, C-65, C-80
39	10.7 q	1.54 s	C-24, C-26	1′	174.0 s[f]		
40	17.9 q	0.57 d (6.5)	C-26, C-27, C-28	2′	68.9 d	4.50 dd (8.4, 4.0)	C-1′, C-3′, C-4′
41	10.7 q	1.52 s	C-28, C-29	3′	40.1 t	2.56 m, 2.66 m	C-1′, C-2′, C-4′
42	10.0 q	1.02 d (7.0)	C-32, C-33, C-34	4′	173.9 s[f]		

[a] ^{1}H and ^{13}C spectra were acquired at 500 and 125 MHz, respectively.
[b] To facilitate spectral comparisons, the numbering scheme is the same as that used originally for **1**;[1] thus, compound **3** does not contain a C-66.
[c] Multiplicity inferred from the DEPT pulse sequence.
[d] Correlation only observed in DMF-d_7.
[e] Assignments may be interchanged.
[f] Assignments may be interchanged.

The aqueous extract (37.5 g) of a Philippines collection of *Ircinia* sp. was fractionated in a manner similar to that described above, to provide 5 mg of a new compound that was given the name chondropsin C (**3**).‡ A molecular formula of $C_{81}H_{131}N_3O_{23}$ was established for **3** by HRFABMS. NMR data sets were obtained in DMF-d_7, to facilitate spectral comparisons with the other chondropsins, and in CD_3OH, as this solvent provided improved spectral dispersion and resolution. This allowed complete assignment of the ^{1}H and ^{13}C NMR resonances for **3** (Table 2). Both the macrocyclic ring and acyclic portions of **3** had NMR signals that corresponded closely with those recorded for compounds **1** and **2**. However, the ^{13}C NMR spectrum of **3** had one less carbonyl resonance, and the OCH_3 group seen in **1** and **2** was missing in **3**. The C-80 and C-81 *gem* dimethyl groups in **3** appeared as a pair of doublets, each coupled to a new methine proton (δ 1.27) at

‡ Compound **3**: white powder; $[\alpha]_D$ +2.7 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 222 (4.66), 228 (4.64), 261 (4.58) nm; IR υ_{max} (film) 3500–3200, 1730, 1699, 1630, 1540, 1208, 1199, 1068, 1021, 958 cm^{-1}; ^{1}H and ^{13}C NMR see Table 2; HRFABMS (CsI-doped) obs. $[M+Cs]^+$, m/z 1646.8165, $C_{81}H_{131}CsN_3O_{23}$ requires 1646.8224.

C-65. COSY and HMBC correlations confirmed this assignment. Thus, **3** lacked the entire methyl ester functionality that terminated the acyclic chain in **1** and **2**. Spectral characteristics of the region around C-73 in **3** closely matched those observed in **2**. Data from DEPT, HSQC, COSY and HMBC experiments unambiguously established the presence of a methylene group at C-73, as seen in **2**. Additional evidence supporting the structure of **3** included an HMBC correlation from H-34 (δ 5.06) to C-1 (δ 172.7), which confirmed that ring closure of the macrolide was effected via esterification with the C-34 oxygen substituent. Attachment of the malic acid residue at C-32 was established by an HMBC correlation between H-32 and the C-4′ ester carbonyl. NOE and coupling constant analyses were consistent with *trans* geometries for all of the olefins in **3**, while a series of 1,3-diaxial NOE interactions defined the relative stereochemistry of the tetrahydropyran ring substituents. Treatment of **3** with diazomethane generated a bis methyl ester derivative (MNa^+, m/z 1565.0), therefore the structure of chondropsin C (**3**) was assigned as shown.

The isolation of 73-deoxychondropsin A (**2**) and chondropsin C (**3**) from two *Ircinia* sponges expands both the known taxonomic distribution and structural scope of the chondropsin family of polyketide-derived macrolide lactams. Compounds **2** and **3** were evaluated for their cytotoxic activity towards melanoma (LOX) and leukemia (MOLT-4) human tumor cell lines in a 2-day in vitro assay.[5] Both compounds exhibited IC_{50}'s of approximately 0.8 and 0.2 ng/mL towards the LOX and MOLT-4 cell lines, respectively.

Acknowledgements

The sponge samples were collected under contract for the National Cancer Institute by the Australian Institute of Marine Science (voucher # Q66C262) and the Coral Reef Research Foundation (voucher # 0CDN3139); vouchers are maintained at the Smithsonian Institution, Washington, DC. We thank G. Cragg and D. Newman (NPB) for collections, T. McCloud for extractions, L. Pannell for MS measurements, and T. Johnson and J. Wilson for cytotoxicity evaluations. This project has been funded in whole or in part with federal funds from the National Cancer Institute, NIH, under contract no. NO1-CO-56000.

References

1. Cantrell, C. C.; Gustafson, K. R.; Cecere, M. R.; Pannell, L. K.; Boyd, M. R. *J. Am. Chem. Soc.* **2000**, *122*, 8825–8829.
2. Boyd, M. R. In *Current Therapy in Oncology*; Niederhuber, J. E., Ed.; Decker: Philadelphia, 1993; pp. 11–22.
3. Boyd, M. R.; Paull, K. D. *Drug Dev. Res.* **1995**, *34*, 91–109.
4. Takada, N.; Sato, H.; Suenaga, K.; Arimoto, H.; Yamada, K.; Ueda, K.; Uemura, D. *Tetrahedron Lett.* **1999**, *40*, 6309–6312.
5. Bokesch, H. R.; Blunt, J. W.; Westergaard, C. K.; Cardellina, II, J. H.; Johnson, T. R.; Michael, J. A.; McKee, T. C.; Hollingshead, M. G.; Boyd, M. R. *J. Nat. Prod.* **1999**, *62*, 633–635.