Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 2003–2006

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

LOBOPHORINS A AND B, NEW ANTIINFLAMMATORY MACROLIDES PRODUCED BY A TROPICAL MARINE BACTERIUM

Zhi-Dong Jiang, Paul R. Jensen, and William Fenical*

Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California at San Diego, La Jolla, CA 92093-0236, U.S.A.

Received 20 March 1999; accepted 28 May 1999

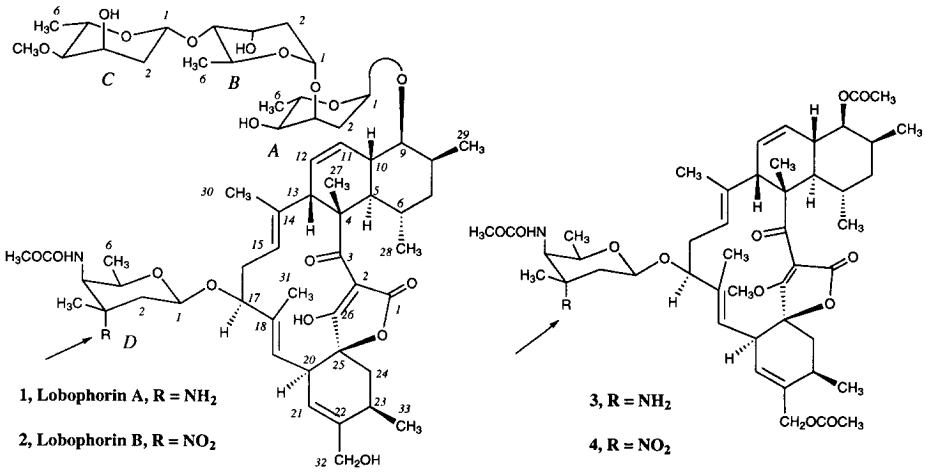
Abstract: Two new antiinflammatory macrolides, lobophorins A and B (**1** and **2**), have been isolated from fermentation broths of a marine bacterium isolated from the surface the Caribbean brown alga *Lobophora variegata* (Dictyotales). The new compounds, distantly related to antibiotics of the kijanimicin class, are potent inhibitors of topical PMA-induced edema in the mouse ear assay when administered either topically or IP.

As part of a continuing program to develop the biomedical potential of marine microorganisms, we have focused considerable attention on the actinomycetes, or filamentious bacteria, which are a rich source of novel compounds in terrestrial environments.[1] In tropical, nutrient-limited oceans, our studies have emphasized surface actinomycetes isolated from the relatively nutrient-rich surfaces of invertebrates and seaweeds.[2] During an expedition to Belize aboard the research vessel Columbus Iselin, we encountered an unusual actinomycete,[3] our strain # CNC-837, isolated from a surface inoculum of the Caribbean brown alga *Lobophora variegata* (Dictyotales).[4] While this organism clearly belonged within the bacterial order Actinomycetales, by FAME analysis, the organism failed to show affinity to any known genus. In saline fermentation this strain produces two new macrolides, lobophorins A and B (**1** and **2**), the aglycones of which are related to the kijanimicin class of microbial antibiotics. Lobophorins A and B are potent antiinflammatory agents when applied topically or administered IP in a whole animal model.

Actinomycete strain # CNB-837 was cultured in 50 L scale using a 65 L fermenter.[5] The filtered broth was extracted by percolation over 2 L coarse C_{18} chromatography resin. After rinsing with distilled water, the resin was eluted with MeOH and the solvent was removed to give a dark-brownish oil. The crude extract was initially fractionated by a silica gel flash chromatography using a gradient of EtOAc/isooctane and MeOH/EtOAc systems. The material eluted with 10% MeOH/EtOAc was purified by RP-C_{18} HPLC using 75% MeOH/H_2O with 0.5% NH_4Ac to give pure lobophorin A (**1**, 40 mg).[6] The material eluted with 100% EtOAc was further purified by silica gel HPLC using 100% EtOAc to give pure lobophorin B (**2**, 800 mg).[6]

Lobophorins A and B were isolated as polar, amorphous solids. High-resolution FABMS analysis showed that lobophorin A possessed the molecular formula $C_{61}H_{92}N_2O_{19}$ while lobophorin B analyzed for $C_{61}H_{90}N_2O_{21}$. Interpretation of their respective ^1H and ^{13}C NMR spectra (Table) showed almost identical structural features were present, including several sugars and a complex, highly oxygenated aglycone. Since both compounds presented broadened NMR spectra, the sugars were cleaved by acid hydrolysis (1 N HCl) to

0960-894X/99/$ - see front matter
PII: S0960-894X(99)00337-6



produce the corresponding aglycones, which were then converted to aglycones C and D (**3** and **4**) by CH_2N_2 methylation and Ac_2O/pyr acetylation. Comparison of their ^{13}C NMR spectra showed that aglycone C (**3**) exhibited a carbon signal at 57.4 ppm, while in aglycone D (**4**) this signal was observed at 90.8 ppm. Other than these differences, the ^{13}C signals of the aglycones were almost identical.[2] Comprehensive NMR analyses performed on aglycones **3** and **4** led to significant insight into the structures of these molecules. Comparison of the NMR data from aglycone **4** with that reported for several known molecules showed that **4** was identical to the aglycone derived by selective acid hydrolysis of the terrestrial antibiotic kijanimicin.[7] However, because the complete NMR assignments for **4** were not available, we used a combination of 2-D NMR techniques, including COSY, HMQC and HMBC methods, to fully assign the NMR data for the aglycones. Using the same comprehensive NMR methods, we were also able to completely assign the NMR data for lobophorins A and B (see NMR Table below). During the spectral analyses, it became apparent that lobophorin B differed only in the sugar-D moiety. On the basis of all data obtained, lobophorin A was assigned an axial amino group at the sugar D-3 position while lobophorin B possessed a nitro group at the same site. Lobophorin B contains one less sugar moiety than kijanimicin and was, coincidentally, reported as a metaperiodate reaction product of kijanimicin.

Despite their structural relationship to kijanimicin and to several related antibiotic macrolide glycosides such as tetrocarcins and chlorothricin,[8a,b] lobophorins A and B (**1** and **2**) do not exhibit significant antibiotic properties. However, lobophorins A and B showed potent antiinflammatory activities in the PMA (Phorbol-Myristate-Acetate)-induced mouse ear edema model. At the normal testing dose of 50 μg/ear topical application, lobophorin A reduced edema by 86%. In the same experiment, lobophorin B reduced edema by 84%. By comparison, the common anti-inflammatory drug, indomethacin (Indocin), showed 72% reduction.[9] More importantly, when administered by intraperitonyl injection (IP), lobophorin B demonstrated *in vivo* activity reducing edema by 34% at a dose of 30 mpK. Based upon the whole animal *in vivo* activities of the lobophorins, studies of their mechanism of action appear warranted.

Table. NMR Assignments for Lobophorins A and B (**1** and **2**)[a]

	Lobophorin A (1)			Lobophorin B (2)	
Compd	^{1}H NMR in δ (mult, *J* in Hz)[b]	^{13}C NMR[b]	^{13}C NMR[c]	^{1}H NMR in δ (mult, *J* in Hz)[c]	^{13}C NMR[c]
1		173.5	167.0		167.0
2		100.0	101.6		101.6
3		201.3	206.2		206.3
4		51.7	50.8		50.8
5	2.32 (m)	44.6	42.9	2.00 (m)	43.0
6	1.65 (m)	31.6	31.1	1.6 (m)	31.2
7	1.58 (m), 1.50 (m)	42.6	41.6	1.6 (m), 1.5 (m)	41.6
8	2.40 (m)	35.1	34.3	2.20 (m)	34.3
9	3.68 (m)	84.6	83.5	3.44 (m)	84.0
10	2.32 (m)	39.3	38.3	2.06 (m)	38.3
11	6.07 (d,10.0)	126.3	125.9	5.72 (d, 10.0)	125.7
12	5.68 (dd, 4.5, 10.0)	128.3	126.1	5.36 (m)	126.5
13	4.51 (m)	51.7	53.0	3.46 (m)	53.1
14		137.2	136.9		138.1
15	5.9 (m)	123.0	123.3	5.17 (m)	123.9
16	2.52 (m)	31.9	29.9	1.70 (m), 2.33 (m)	29.9
17	4.44 (m)	79.8	77.6	4.20 (m)	78.1
18		136.0	135.7		135.1
19	5.85 (d, 10.5)	121.6	119.0	5.13 (m)	118.6
20	3.86 (br d, 10.0)	41.2	40.0	3.60 (d, 10.0)	40.0
21	6.16 (br s)	123.9	121.4	5.40 (br s)	121.3
22		142.5	141.2		141.3
23	2.98 (br p)	28.3	27.7	2.63 (br p, 7.0)	28.0
24	2.70 (dd, 7.5, 14.5), 2.03 (d, 14.5)	36.0	35.5	2.36 (m), 1.80 (d, 14.0)	35.4
25		83.7	83.2		83.2
26		200.9	201.6		201.7
27	1.95 (s)	15.5	15.0	1.60 (s)	15.0
28	0.72 (d, 6.5)	22.9	22.1	0.64 (d, 4.5)	22.2
29	1.34 (d, 7.0)	14.6	14.1	1.09 (d, 6.5)	14.1
30	1.60 (s)	14.6	15.0	1.40 (s)	15.2
31	1.43 (s)	14.9	13.7	1.30 (s)	13.7
32	4.73 (d, 12.5), 4.66 (d, 12.5)	65.0	64.8	4.15 (m)	64.7
33	1.52 (d, 6.0)	20.2	20.1	1.29 (d, 7.0)	20.2
D-1	4.78 (dd, 1.5, 10.0)	98.4	96.5	4.67 (dd, 2.0, 10.0)	97.7
D-2	1.80 (dd, 10.0, 15.0), 2.50 (m)	36 0	35.2	1.40 (m), 1.58 (m)	39.9
D-3		91.6	90.8		53.0
D-4	4.85 (d, 10.0)	54.7	53.6	3.15 (d, 10.0)	57.7
D-5	3.58 (q, 7.5)	69.1	69.0	3.76 (m)	68.0
D-6	1.20 (d, 6.5)	17.3	16.8	1.10 (d, 6.5)	17.9
D-3 CH_3	1.46 (s)	25.3	25.1	1.17 (s)	30.0
D-4 NH	8.90 (d, 10.0)			5.20 (d, 10.0)	
D-4 C=O		158.8	157.6		157.5
D-4 OCH_3	3.81 (s)	52.3	52.6	3.67 (s)	52.2
A-1	5.08 (d, 4.5)	98.4	97.9	4.78 (d, 4.5)	98.0
A-2	1.90 (m), 2.62 (br d,13.0)	30.3	30.6	1.70 (m), 2.33 (m)	30.2
A-3	4.42 (m)	67.2	66.2	4.0 (m)	66.3
A-4	3.68 (m)	72.3	71.1	3.23 (dd, 2.0, 10.0)	71.7
A-5	4.52 (m)	65.4	64.8	4.20 (m)	64.9
A-6	1.52 (d, 6.0)	18.6	18.2	1.30 (d, 6.5)	18.3
B-1	5.56 (d,3.5)	92.0	91.0	5.13 (d, 3.0)	90.9
B-2	2.10 (m), 2.50 (m)	36.0	34.0	1.88 (dt, 15.0, 3.5), 2.12 (m)	34.0
B-3	4.64 (m)	67.2	65.3	3.98 (m)	65.3
B-4	3.55 (dd, 2.5, 10.0)	82.6	82.1	3.23 (dd, 10.0, 2.0)	82.1
B-5	4.60 (dq, 10.0, 6.5)	63.0	61.9	4.0 (m)	61.9
B-6	1.35 (d, 6.0)	17.9	17.8	1.20 (d, 6.5)	17.7
C-1	5.40 (dd, 1.5, 10.0)	100.0	98.2	4.91 (dd, 10.0, 2.0)	98.1
C-2	1.88 (m), 2.40 (m)	38.7	36.6	1.68 (m), 2.15 (m)	36.6
C-3	4.46 (m)	63.2	63.7	4.24 (m)	63.7
C-4	2.93 (dd, 3.0, 9.5)	83.5	82.1	2.85 (dd, 10.0, 3.0)	82.1
C-5	4.25 (dq, 9.5, 6.5)	68.6	68.2	4.20 (m)	68.3
C-6	1.38 (d, 6.5)	18.6	17.8	1.24 (d, 6.5)	17.3
C-4 OCH_3	3.31 (s)	56.3	57.2	3.41 (s)	57.3

[a]All assignments were made on the basis of ^{1}H-^{1}H COSY, DEPT, HMQC and HMBC experiments.
[b]Spectra were measured in pyridine-d_5.
[c]Spectra were measured in $CDCl_3$.

Acknowledgements: This research was supported by the National Institutes of Health, National Cancer Institute, under grant CA44848. We thank Professor Robert S. Jacobs and his students, University of California-Santa Barbara, for providing *in vitro* and *in vivo* antiinflammatory testing data.

References and Notes

1. Higton, A. A.; Roberts, A. D. In *Dictionary of Antibiotics and Related Substances*; Bycroft, B. Y. , Ed.; Chapman and Hall: New York, 1988; xviii, p 944.
2. Moore, B. S.; Trischman, J. A.; Seng, D.; Kho, D.; Jensen, P. R.; Fenical, W. *J. Org. Chem.* **1999**, *64*, 1145.
3. The isolate, strain CNB-837, was illustrated to be a member of the Order Actinomycetales by Fatty Acid Methyl Ester Analysis (FAME) (Microbial Analysis Lab., Northfield, VT) and by morphological evaluation. The strain showed a poor similarity index (0.138) with its closest genus, *Streptomyces.* The poor correlation may indicate that this is a unique microorganism. The strain produced branched hyphae with areal mycelia and grey spores.
4. The brown alga *Lobophora variegata* was collected –15 m from a patch reef area near Gallow's Point, Belize, in 1991. One cm^2 of the alga's surface was swabbed with a sterile cotton applicator and the cotton tip was transferred to sterile seawater, vortex mixed, serially diluted, and the liquid plated onto a solid agar medium containing starch 0.25%, yeast extract 0.05%, peptone 0.1%, sodium glycerol phosphate 0.01%, seawater 75%, and de-ionized water 25%. The medium also contined cycloheximide (75 μg/mL, added after autoclaving) to suppress fungal contamination.
5. Strain CNB-837 was cultured using Medium A1 (soluble starch 1%, yeast extract 0.4%, peptone 0.2%, seawater 75%, deionized water 25%) at 25 °C and at a shaker speed of 230 rpm for 7 days.
6. **For lobophorin A (1)**: $[\alpha]_D^{22}$ –175° (*c* 0.28, MeOH). UV λ_{max} nm in MeOH (ε): 204 (3.6×10^4), 241 (1.3×10^4), 268 (1.1×10^4) and 280 (1.0×10^4). IR ν_{max} (neat) cm^{-1}: 3415, 2917, 1707, 1623, 1543, 1411, 1377, 1093, 1067, 1005, 917 and 726. HR FABMS gave $[M+H]^+$ *m/z* 1157.6357, $C_{61}H_{93}N_2O_{19}$ requires 1157.6372, –1.3 ppm dev. Fragmentation *m/z* (rel. int.): 885 (3), 753 (2), 569 (4), 525 (5), 257 (6), 201 (11), 184 (24). **For lobophorin B (2)**: $[\alpha]_D^{22}$ 104° (*c* 2.0, MeOH). UV λ_{max} nm in MeOH (ε): 204 (4.0×10^4), 239 (1.4×10^4), 265 (1.1×10^4) and 277 (1.0×10^4). HRFABMS: $[M+Na^+]$ *m/z* 1209.5957, $C_{61}H_{90}N_2O_{21}Na$ requires 1209.5933, 1.9 ppm dev. NMR data for aglycones: **For aglycone 3**: ^{13}C NMR ($CDCl_3$, 50 MHz): δ 198.7, 189.8, 171.1, 170.5, 169.9, 168.8, 157.4, 138.4, 137.6, 135.7, 128.6, 127.9, 123.9, 122.3, 118.7, 106.8, 97.8, 82.6, 78.8, 78.4, 68.0, 67.4, 63.7, 57.4, 53.9, 53.7, 52.3, 50.6, 44.3, 41.2, 40.0, 37.4, 37.1, 36.1, 31.4, 29.0, 24.6, 24.3, 23.8, 21.3, 21.1, 20.0, 17.3, 14.7, 14.3, 14.0, 13.8. **For aglycone 4**: ^{13}C NMR ($CDCl_3$, 50 MHz): δ 198.7, 190.0, 170.9, 170.5, 168.9, 157.3, 138.0, 136.1, 127.7, 124.9, 124.1, 121.8, 119.1, 106.7, 97.5, 90.8, 82.9, 79.0, 78.4, 68.8, 66.1, 63.8, 53.8, 52.7, 50.5, 44.3, 41.2, 39.9, 37.2, 36.0, 35.7, 31.4, 28.0, 25.3, 24.3, 21.2, 20.9, 19.8, 17.0, 14.6, 14.5, 14.1, 13.9.
7. Mallams, A. K.; Puar, M. S.; Rossman, R. R.; McPhail, A. T.; Macfarlane, R. D.; Stephens, R. L. *J. Chem. Soc. Perkin Trans. 1* **1983**, 1497.
8. (a) Hirayama, N.; Kasai, M.; Shirahata, K.; Ohashi, Y.; Sasada, Y. *Bull. Chem. Soc. Jpn.* **1982**, *55*, 2984. (b) Muntwyler, R.; Keller-Schierlein, W. *Helv. Chim. Acta* **1972**, *55*, 2071.
9. Jacobson, P. B.; Jacobs, R. S. *J. Pharmacol. Exp. Ther.* **1992**, *262*, 866.