Pergamon

Tetrahedron Letters, Vol. 37, No. 9, pp. 1327-1330, 1996
Copyright © 1996 Elsevier Science Ltd
Printed in Great Britain. All rights reserved
0040-4039/96 $15.00 + 0.00

S0040-4039(96)00014-7

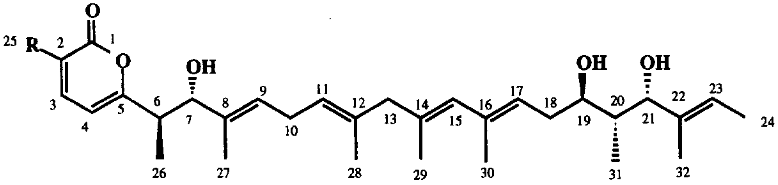
LAGUNAPYRONES A-C: CYTOTOXIC ACETOGENINS OF A NEW SKELETAL CLASS FROM A MARINE SEDIMENT BACTERIUM

Thomas Lindel[1], Paul R. Jensen, and William Fenical*

Scripps Institution of Oceanography
University of California-San Diego
La Jolla, CA 92093-0236

Abstract-: The structures of the lagunapyrones A-C (**1-3**), novel, cytotoxic α-pyrones, produced in fermentation by a marine bacterium, have been assigned on the basis of comprehensive spectroscopic analyses. Transformation of lagunapyrone B (**2**) to its [1', 3'-$^{13}C_2$]-labelled acetonide allowed the relative stereochemistry of the flexible 1,3-diol moiety to be determined.

Marine bacteria are considered to play a central role as symbionts of most marine invertebrates and also represent one of the most novel biomedical resources remaining to be explored.[2] As part of an investigation of the secondary metabolism of estuarine actinomycetes, we report here the isolation and structural elucidation of the lagunapyrones A-C (**1-3**), closely related α-pyrones functionalized by a highly methyl-branched C_{19} side chain. Pyrones **1-3** were produced, in a seawater-based medium, by an unidentified marine actinomycete (culture CNB-984) isolated from sediment collected in the Agua Hedionda Lagoon in Carlsbad, California.[3] Lagunapyrone B (**2**) shows modest *in vitro* cytotoxicity, ED_{50} = 3.5 μg/ml, against the human colon cancer cell line HCT-116.



1, R = CH_3

2, R = $\overset{25}{CH_2}\overset{25a}{CH_2}\overset{25b}{CH_3}$

3, R = $CH_2CH_2CH_2\overset{25c}{CH_3}$

The crude ethyl acetate extract of a 20 L culture of the marine actinomycete (CNB-984) was partitioned between isooctane and methanol. The methanol fraction was again partitioned between ethyl acetate and water to obtain 800 mg of material from the ethyl acetate phase. It was possible to separate lagunapyrone A (**1**, 10 mg) from its higher homologues and from piericidin[4] by size exclusion chromatography (Sephadex LH20, methanol), while the purification of lagunapyrone B (**2**, 25 mg) and C (**3**, 5 mg) required repeated normal phase TLC or HPLC (silica gel, ethyl acetate/*n*-heptane, 3:2).

The molecular formula for lagunapyrone B (**2**, $C_{34}H_{52}O_5$, by HRFABMS) indicated nine degrees of unsaturation. Acetylation, and isolation of a triacetate, proved the presence of three hydroxyl groups.[5b] On the basis of the ^{13}C NMR/DEPT spectral data (see Table), the remaining two oxygen atoms were concluded to be attached to two quaternary, sp^2-hybridized carbon atoms (163.9 and 164.6 ppm). Together with the ^1H NMR, IR and UV spectral data[5b], ten convincing HMBC correlations[6] (see Table) established the α-pyrone moiety. The constitution of the highly methyl-branched side-chain was secured by more than 60 additional HMBC correlations. Assignment of all five trisubstituted double bonds as possessing *E*-configurations was accomplished by analysis of NOESY correlations and by methyl group ^{13}C NMR chemical shifts, all of which were at higher field than 18 ppm.

Table. ^{1}H and ^{13}C NMR, and HMBC data, (500 MHz, $CDCl_3$) for lagunapyrone B (2).

#	^{13}C NMR a)	^{1}H NMR	HMBC correlations b)
1	163.9 (C)		
2	126.9 (C)		
3	139.0 (CH)	7.04 (d, 6.8 Hz)	C-5, C-25
4	103.8 (CH)	6.03 (d, 6.8 Hz)	C-2, C-5, C-6
5	164.6 (C)		
6	42.3 (CH)	2.69 (m)	C-4, C-7, C-26
7	79.7 (CH)	4.20 (d, 9.8 Hz)	C-6, C-8, C-9, C-26, C-27
8	134.3 (C)		
9	129.1 (CH)	5.47 (t, 6.4 Hz)	C-7, C-10, C-11, C-27
10	26.8 (CH2)	2.78 (dd, 6.4, 6.4 Hz)	C-8, C-9, C-11, C-12
11	124.0 (CH)	5.17 (t, 6.4 Hz)	C-9, C-10, C-13, C-28
12	134.1 (C)		
13	51.0 (CH2)	2.68 (s)	C-11, C-12, C-15, C-28, C-29
14	136.0 (C)		
15	130.3 (CH)	5.69 (s)	C-13, C-17, C-29
16	134.1 (C)		
17	124.7 (CH)	5.32 (t, 7.3 Hz)	C-15, C-30
18	34.1 (CH2)	2.40 (m)	C-17, C-19, C-20
19	75.2 (CH)	3.71 (m)	C-17, C-21, C-31
20	39.3 (CH)	1.79 (m)	none
21	75.5 (CH)	4.38 (d, 4.3 Hz)	C-19, C-20, C-22, C-23, C-31, C-32
22	136.1 (C)		
23	118.7 (CH)	5.54 (q, 6.3 Hz)	C-21, C-24
24	13.6 (CH3)	1.65 (d, 6.3 Hz)	C-22, C-23
25	32.4 (CH2)	2.40 (t, 7.3 Hz)	C-1, C-2, C-3, C-25a, C-25b
25a	21.0 (CH2)	1.60 (tq, 7.3, 7.3 Hz)	none
25b	13.8 (CH3)	0.96 (t, 7.3 Hz)	C-25, C-25a
26	15.6 (CH3)	1.07 (d, 7.3 Hz)	C-5, C-6, C-7
27	10.4 (CH3)	1.66 (s)	C-9
28	15.7 (CH3)	1.57 (s)	C-11, C-12, C-13
29	17.4 (CH3)	1.67 (s)	C-13, C-15
30	17.3 (CH3)	1.78 (s)	C-15, C-16, C-17
31	10.6 (CH3)	1.56 (s)	C-21, C-22, C-23

a) Numbers of attached protons were determined by DEPT experiments.

b) HMBC experiment was optimized for $J = 8$ Hz.

Assignment of the relative stereochemistry at C-6 and C-7 was accomplished by computer analysis of the vicinal proton coupling constants, and by comparison of these values with two isomeric synthetic analogs in which phenyl is substituted for the α-pyrone.[7] The coupling constant observed ($J_{H6,\,H7}$ = 9.8 Hz) agreed well with the $6\boldsymbol{R}^*$, $7\boldsymbol{S}^*$ synthetic isomer, thus **2** was assigned this configuration.[7] Nuclear Overhauser enhancements between the C-26 and C-27 methyl groups, between H-6 and the C-27 methyl group, and between H-7 and H-9 also supported that conclusion. Lagunapyrone B (**2**) was converted to its C-19 - C-21 ^{13}C-labelled acetonide by treatment with [1, 3-$^{13}C_2$] acetone in dry CH_2Cl_2/toluene (1:1) in the presence of *p*-toluenesulfonic acid, or alternatively, employing the Noyori procedure.[8] The ^{13}C NMR spectrum of the acetonide clearly showed two methyl doublets at 24.0 and 25.1 ppm ($^2J_{C,\,C}$ = 23.4 Hz), indicating a *twist-boat* conformation of the 1, 3-dioxolane ring.[9] This, in combination with

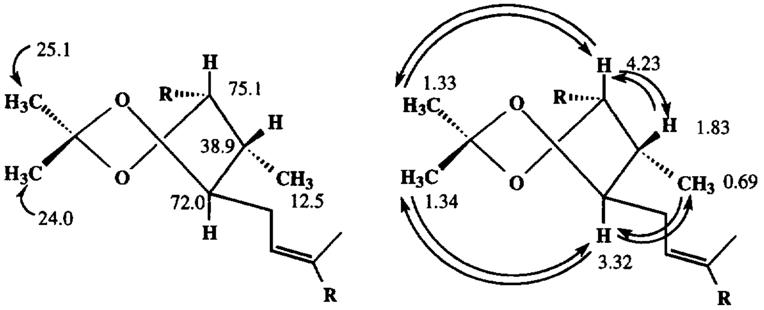


Figure. ^{13}C and ^{1}H NMR shifts, with selected ^{1}H NMR nuclear Overhauser enhancements for the C-19, C-21 acetonide derivative of lagunapyrone B.

the NOE enhancements shown in the Figure, led to assignment of the relative configuration of the 1, 3-diol substructure of lagunapyrone B as $19R^*$, $20R^*$and $21S^*$. *It is important to point out that the C-6, C-7 stereochemistry cannot be related to the relative stereochemistry assigned at C-19 through C-21. Thus, the alternative* $6S^*$, $7R^*$, $19R^*$, $20R^*$, $21S^*$ *configuration may also be assigned.*

Instead of an *n*-propyl group, lagunapyrones A (**1**) and C (**3**) were found to possess methyl and *n*-butyl substituents at the 2-position of the pyrone ring.[5a,c] The ^{1}H NMR spectrum of lagunapyrone A (**1**) lacked the methyl triplet at δ 0.96 and showed an additional singlet at δ 2.07, while the methyl triplet (C-25c, at δ 0.93) of lagunapyrone C (**3**) showed a small high field shift in comparison to the corresponding signal of **2**. On the basis of COSY experiments, the saturated linear chain of four carbon atoms in **3** was assigned. As indicated by all other NMR chemical shifts, coupling constants, 2D NMR correlations, and optical rotations, the side chains of **1** and **3** were confidently assigned the same composition and relative stereochemistry as that of **2**.

The carbon skeleton of the lagunapyrones has not been previously observed. Biosynthetically, these compounds could be derived by the condensation of acetate and/or propionate units, or by a combination including methylation of the carbon chain through the methionine pathway. A structurally similar metabolite, leptomycin, isolated from a *Streptomyces* sp. inhibits the proliferation of *Schizosaccharomyces pombe* in both the G1 and the G2 phases of the cell cycle.[10]

ACKNOWLEDGEMENTS

This research is a result of financial support from the National Institutes of Health, National Cancer Institute, under grant CA50760. T. L. wishes to thank the Deutsche Forschungsgemeinschaft for a research stipend (II C 2 - Li 597/1-1) and C. Kauffman for technical assistance.

REFERENCES AND NOTES

1. Current address: Pharmazeutisch-Chemisches Institut der Universität, Im Neuenheimer Feld 364, D-69120 Heidelberg, Germany.
2. Fenical, W. *Chem. Rev.* **1993**, *93*, 1673-1683.
3. The bacterium CNB-984, an actinomycete with non-fragmented, branched substrate mycelia, could not be identified by fatty acid analysis (Microcheck, Inc., Northfield, VT). The strain produced lagunapyrones in a seawater-based medium (1% starch, 0.4% yeast extract, 0.2% peptone) while shaking at 230 rpm. The strain grew poorly and lagunapyrones were not produced when seawater was replaced with deionized water in the culture medium.

4. Yoshida, S.; Shiraishi, S.; Fujita, K.; Takahashi, N. *Tetrahedron Lett.* **1975**, *16*, 1863-1866.

5. **a)** Spectral data for lagunapyrone A (**1**): optical rotation: $[\alpha]_D^{20}$ = + 40.1° (*c* = 1.8, MeOH). - UV (CH_2Cl_2): λ_{max} (log ε) = 225 nm (3.978), 298 (3.816). - IR (neat): 3423 (br.), 2977, 2918, 1697, 1639, 1576, 1450, 1382, 1110, 1081, 1042, 1008 cm^{-1}. - ^1H NMR (500 MHz, $CDCl_3$): δ 0.89 (d, *J* = 7.0 Hz, 3H), 1.06 (d, *J* = 7.0 Hz, 3H), 1.56 (2s, 6H), 1.64 (d, *J* = 7.0 Hz, 3H), 1.65 (s, 3H), 1.66 (s, 3H), 1.77 (s, 3H), 1.79 (m, 1H), 2.07 (s, 3H), 2.35-2.45 (2m, 2H), 2.40 (t, *J* = 8.0 Hz, 2H), 2.67 (s, 2H), 2.69 (m, 1H), 2.77 (dd, *J* = 7.7, 7.7 Hz, 2H), 3.72 (m, 1H), 4.20 (d, *J* = 9.5 Hz, 1H), 4.38 (m, 1H), 5.16 (t, *J* = 7.0 Hz, 1H), 5.31 (dd, *J* = 6.7, 6.7 Hz, 1H), 5.47 (t, *J* = 6.7 Hz, 1H), 5.54 (d, *J* = 6.7 Hz, 1H), 5.69 (s, 1H), 6.02 (d, *J* = 6.8 Hz, 1H), 7.08 (d, *J* = 6.8 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$,: δ 10.4 (CH3), 10.6 (CH3), 13.0 (CH3), 13.5 (CH3), 15.6 (CH3), 15.7 (CH3), 16.6 (CH3), 17.3 (CH3), 17.3 (CH3), 26.8 (CH2), 34.2 (CH2), 42.3 (CH), 51.0 (CH), 75.2 (CH), 75.5 (CH), 79.9 (CH), 103.8 (CH), 118.7 (CH), 122.8 (C), 124.0 (C), 124.7 (CH), 129.1 (CH), 130.3 (CH), 134.1 (2s), 134.3 (C), 136.1 (2s), 139.8 (CH), 164.3 (C), 164.9 (C). - MS (pos. FAB, NBA/NaCl): *m/z* (%) = 535 (63) $[M + Na]^+$, 513 (7) $[M + H]^+$, 477 (9), 459 (10), 138 (100) [matrix]. - HRFABMS: calcd. for $[C_{32}H_{48}O_5Na]^+$: 535.3399, found 535.3394. **b)** Spectral data for lagunapyrone B (**2**): optical rotation: $[\alpha]_D^{20}$ = + 10.9° (*c* = 3.7, CH_2Cl_2). - UV (CH_2Cl_2): λ_{max} (log ε) = 229 nm (3.950), 298 (3.722). - IR (neat): 3401(br.), 2963, 2924, 1698 (s), 1634, 1576, 1454, 1381, 1114, 1084, 1041, 1007 cm^{-1} . - ^1H NMR, ^{13}C NMR: see Table. - MS (pos. FAB, NBA/NaCl): *m/z* (%) = 563 (14) $[M + Na]^+$, 541 (12) $[M + H]^+$, 505 (16), 154 (100) [matrix]. HRFABMS: calcd. for $[C_{34}H_{52}O_5H]^+$: 541.3893, found 541.3826; Lagunapyrone B triacetate: calcd. for $[C_{40}H_{58}O_8Na]^+$: 689.4029, found 689.3997; Lagunapyrone B acetonide: ^1H NMR (500 MHz, $CDCl_3$): δ 0.69 (d, *J* = 7.3 Hz, 3H), 0.95 (t, *J* = 7.3 Hz, 3H), 1.05 (d, *J* = 7.3 Hz, 3H), 1.33 (s, 3H), 1.34 (s, 3H), 1.52 (s, 3H), 1.57 (s, 3H), 1.59 (m, 2H), 1.65 (s, 3H), 1.66 (d, *J* = 6.3 Hz, 3H), 1.67 (s, 3H), 1.72 (s, 3H), 1.83 (m, 1H), 2.30 (m, 1H), 2.34 (m, 1H), 2.43 (t, 2H, *J* = 6.8 Hz), 2.67 (s, 2H), 2.69 (m, 1H), 2.77 (dd, *J* = 6.8, 6.8 Hz, 2H), 3.32 (m, 1H), 4.20 (d, *J* = 9.8 Hz, 1H), 4.23 (d, *J* = 4.4 Hz, 1H), 5.16 (t, *J* = 6.8 Hz, 1H), 5.34 (dd, *J* = 5.8, 5.8 Hz, 1H), 5.48 (t, *J* = 6.8 Hz, 1H), 5.49 (q, *J* = 6.3 Hz, 1H), 5.68 (s, 1H), 6.03 (d, *J* = 6.3 Hz, 1H), 7.04 (d, *J* = 6.3 Hz, 1H). - ^{13}C NMR (100 MHz, $CDCl_3$): δ 10.4 (CH3), 12.5 (CH3), 12.7 (CH3), 13.7 (CH3), 13.8 (CH3), 15.5 (CH3), 15.7 (CH3), 17.1 (CH3), 17.3 (CH3), 21.1 (CH2), 24.0 (CH3), 25.1 (CH3), 26.8 (CH2), 32.4 (CH2), 33.6 (CH2), 38.9 (CH), 42.3 (CH), 51.0 (CH2), 72.0 (CH), 75.1 (CH), 79.7 (CH), 100.7 (C), 103.7 (CH), 118.0 (CH), 123.8 (CH), 125.4 (CH), 126.9 (C), 129.1 (CH), 130.5 (CH), 132.7 (C), 134.1 (C), 134.2 (C), 136.0 (C), 136.1 (C), 139.0 (CH), 163.9 (C), 164.6 (C). HRFABMS calcd. ($[C_{37}H_{56}O_5Na]^{+)}$: 603.4025, found 603.3997. **c)** Spectral data for lagunapyrone C (**3**): optical rotation: $[\alpha]_D^{20}$ = + 46.0° (*c* = 1.5, MeOH). - UV (CH_2Cl_2): λ_{max} (log ε) = 227 nm (4.021), 298 (3.862). - IR (neat): 3394 (br.), 2957, 2928, 1697, 1639, 1571, 1450, 1377, 1081, 1013 cm^{-1}; - ^1H NMR (500 MHz, CD_3OD): δ 0.87 (d, *J* = 7.3 Hz, 3H), 0.94 (t, *J* = 7.3 Hz, 3H), 1.03 (d, *J* = 6.8 Hz, 3H), 1.36 (m, 2H), 1.53 (m, 2H), 1.56 (s, 3H), 1.57 (s, 3H), 1.61 (d, *J* = 6.8 Hz, 3H), 1.66 (2s, 6H), 1.71 (s, 3H), 1.78 (m, 1H), 2.18-2.36 (2m, 2H), 2.40 (t, *J* = 8.8 Hz, 2H), 2.67 (s, 2H), 2.72 (dq, *J* = 11.7, 8.8 Hz, 1H), 2.80 (dd, *J* = 7.3, 7.3 Hz, 2H), 3.68 (m, 1H), 4.08 (d, *J* = 9.8 Hz), 4.14 (d, *J* = 5.4 Hz, 1H), 5.20 (t, *J* = 6.9 Hz, 1H), 5.34 (t, *J* = 6.8 Hz, 1H), 5.44 (t, *J* = 7.3 Hz, 1H), 5.50 (q, *J* = 6.8 Hz, 1H), 5.67 (s, 1H), 6.18 (d, *J* = 6.8 Hz, 1H), 7.24 (d, *J* = 6.8 Hz, 1H). ^{13}C NMR (50 MHz, CD_3OD: δ 10.3 (CH3), 10.7 (CH3), 12.7 (CH3), 13.1 (CH3), 14.2 (CH3), 15.6 (CH3), 16.3 (CH3), 17.5 (CH3), 17.5 (CH3), 23.4 (CH2), 27.7 (CH2), 31.0 (CH2), 31.4 (CH2), 33.3 (CH2), 41.9 (CH), 43.4 (CH), 51.9 (CH2), 74.5 (CH), 77.7 (CH), 80.8 (CH), 105.7 (CH), 120.5 (CH), 125.3 (CH), 126.3 (C), 126.9 (C), 129.0 (CH), 131.8 (CH), 134.4 (C), 134.7 (C), 135.8 (C), 136.9 (C), 137.2 (C), 141.9 (CH), 166.9 (C), 167.6 (C). - HRFABMS: calcd. for $[C_{35}H_{54}O_5Na]^+$: 577.3869, found 577.3852.

6. Summers, M. F.; Marzilli, L. G.; Bax, *A. J. Am. Chem. Soc.* **1986**, *108*, 4285-4294; Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2093-2094.

7. MMX calculations: PCMODEL V 5.0, 2nd ed., Serena Software, Bloomington (Indiana), **1994**; see also: Fleming, I.; Lewis, J. L. *J. Chem. Soc . Perkin Trans.* **1992**, *1*, 3267-3275.

8. Tsunoda, T.; Suzuki, M.; Noyori, R. *Tetrahedron Lett.* **1980**, *21*,1357-1358.

9. Rychnovsky, S. D.; Skalitzky, D. J. *Tetrahedron Lett.* **1990**, *31*, 945-948; Rychnovsky, S. D.; Rogers, B.; Yang, G. *J. Org. Chem.*. **1993**, *58*, 3511-3515.

10. Hamamoto, T.; Seto, H.; Beppu, T. *J. Antibiot.* **1983**, *36*, 646-650; Yoshida, M.; Nishikawa, M.; Nishi, K.; Abe, K.; Horinouchi, S.; Beppu, T. *Exp. Cell Res.* **1990**, *187*, 150-156; Hosokawa, N.; Iinuma, H.; Naganawa, H.; Hamada, M.; Takeuchi, T. *J. Antibiot.* **1993**, *46*, 676-678.

(*Received in USA* 31 *October* 1995; *revised* 13 *December* 1995; *accepted* 15 *December* 1995)