Lituarines A, B, and C: A New Class of Macrocyclic Lactones from the New Caledonian Sea Pen *Lituaria Australasiae*

Jean-Pierre Vidal,* Roger Escale, Jean-Pierre Girard, and Jean-Claude Rossi

URA CNRS 1111, Université de Montpellier I, Faculté de Pharmacie, 15 Avenue Charles Flahault, 34060 Montpellier Cédex, France

Jean-Marie Chantraine

Institut Français de Recherche Scientifique pour le Développement en Coopération, ORSTOM, B.P.A5 Nouméa Cédex, Nouvelle Calédonie

André Aumelas

Centre CNRS-INSERM de Pharmacologie - Endocrinologie, Rue de la Cardonille, 34094 Montpellier Cédex 5, France

Received April 16, 1992 (Revised Manuscript Received July 16, 1992)

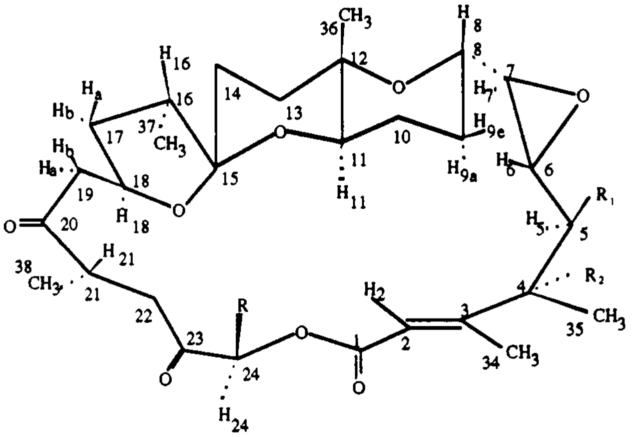
Three new macrocyclic lactones, lituarines A, B, and C, were isolated from the New Caledonian sea pen, *Lituaria australasiae*. Structure elucidation of these compounds was determined through NMR spectroscopic techniques. Lituarines are the first macrocyclic lactones of marine origin exhibiting two coupled tetrahydropyran moieties. Antifungal, cytotoxic and antineoplastic activities were found for these substances.

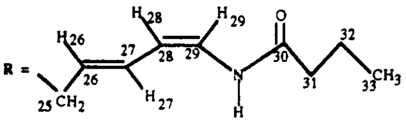
The Coelenterates have been extensively investigated for biological activity, especially the order Alcyonacea (soft corals) and Gorgonacea. Although many chemical studies on the Pennatulacea order (sea pens) have been carried out, the Veretillidae family has been comparatively little investigated.[1] The metabolites that have been isolated from sea pens are all diterpenes.[2]

In the course of our survey on physiologically active compounds[3-4] in marine organisms, we found that some sea pen members contain potentially important antineoplastic constituents.

Bioassay-guided isolation yielded three new 25-membered-ring lactones **1**, **2**, and **3**, named lituarines A, B, and C, respectively, from extracts of the New Caledonian sea pen *Lituaria australasiae*.[5] This paper deals with the isolation and structure elucidation of these compounds.

The ethanol/water (80/20) extract of the sea pen was partitioned between water and CH_2Cl_2. The CH_2Cl_2 phase was fractionated by flash chromatography on silica gel, followed by reversed-phase HPLC to afford lituarine A ($0.16 \times 10^{-3}\%$ based on wet weight), lituarine B ($0.08 \times 10^{-3}\%$), and lituarine C ($0.19 \times 10^{-3}\%$). These new compounds have respectively the following molecular formulas of $C_{38}H_{55}O_9N$, $C_{40}H_{57}O_{12}N$, and $C_{38}H_{55}O_{11}N$. They have been established by their FAB mass spectra, their microanalyses, and their ^1H and ^{13}C NMR spectra. The infrared spectra revealed the presence of hydroxyl groups (3450–3350 cm^{-1}), ethylenic bonds (3060 cm^{-1}), and several functions: amide, ester, and ketone carbonyl (1750–1670, 1650, 1620 cm^{-1}). The FAB spectrum of the lituarine C solubilized in CD_3OD reveals the presence of two exchangeable protons at m/z 704 $(M + 2 + H)^+$. Its acetylation leads to a monoacetylated compound. As lituarines could not be obtained in crystalline state suitable for X-ray crystal structure determination, unequivocal assignment of structures **1**, **2**, and **3** was performed by ^1H and ^{13}C NMR studies (Table I). Two-dimensional NMR experiments of COSY, NOESY, and ROESY (350 ms) were widely used to establish scalar and dipolar ^1H–^1H connectivities. ^1H–^{13}C correlations were obtained with HMQC and HMBC experiments.





	R_1	R_2
Lituarine A	H	H
Lituarine B	$-O-\overset{39}{C}(=O)-\overset{40}{C}H_3$	OH
Lituarine C	OH	OH

As shown by ^{13}C NMR, these compounds, which display the same basic structural features, are characterized by the presence of four quaternary carbons (C3, C12, C15, and C4) for lituarines B and C, and three quaternary carbons (C3, C12, and C15) for lituarine A, six methyl groups, two ketone carbonyl groups (C20 and C23), two carbonyl groups: ester (C1) and amide (C30), respectively, and three double bonds ($\Delta^{2(3)}$, $\Delta^{26(27)}$, and $\Delta^{28(29)}$). The data account for 7 of the 12 degrees of unsaturation, and therefore, the carbon skeleton consists of five rings among which one would be an oxirane (C6, C7). The assignment of all hydrogen-bearing carbons was established first by a DEPT spectrum and second by the ^1H-detected multiple quantum coherence (HMQC) experiment.[6] To make the interpre-

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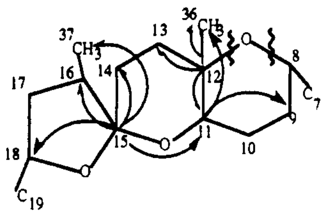
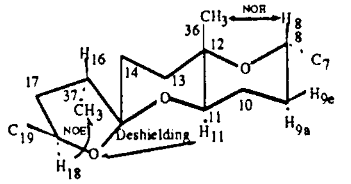
Scheme I



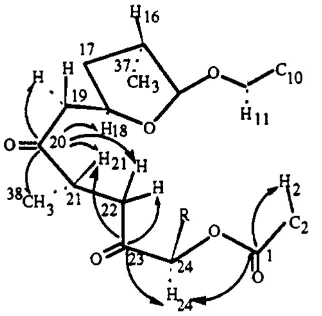
Partial Structure a

Scheme II



Partial Structure a

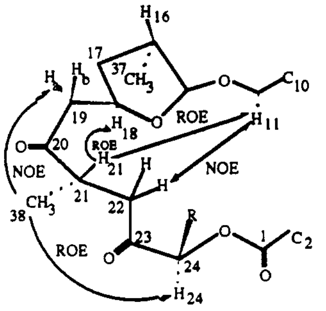
Scheme III



HMBC Partial Structure b

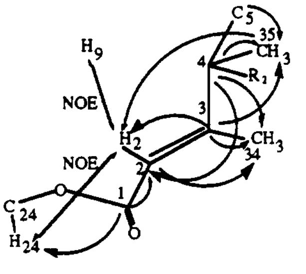
tation easier, we divided the molecule into five partial structures (**a**–**e**) which are linked between them by ^{1}H–^{1}H COSY as well as by long-range ^{1}H–^{13}C correlations inferred from an HMBC experiment. The following results correspond to those of lituarine C. The attribution of the C8–C19 motif (partial structure **a**) (Scheme I) was determined by vicinal couplings[7] and by tracing cross-peaks in the ^{1}H-detected heteronuclear multiple bond correlation (HMBC) spectrum.[8] Elements of this partial structure are observed in nigericine[9] (spiro dioxo quaternary carbon C15 at 107.4 ppm) and in thyrsiferol[10] (quaternary tetrahydropyran carbon C12 at 71.6 ppm). Thus, lituarines are the first macrocyclic lactones of marine origin exhibiting two coupled tetrahydropyran moieties. The trans junction and the chair conformations of the tetrahydropyran cycles are suggested by the coupling constants values ($J_{10,11}$ = 11.8 Hz = J_{aa}, $J_{10,11}$ = 4.3 Hz = J_{ae}, $J_{8,9}$ = 12.7 Hz = J_{aa}, $J_{8,9}$ = 2.7 Hz = J_{ae}) and by the H8–H36 cross-peak observed in the NOESY spectrum (Scheme II).

Scheme IV



NOESY Partial Structure b

Scheme V



HMBC/NOESY

Partial Structure c

Furthermore, this NOE effect determines the C8–O–C12 portion. The stereochemistry of the tetrahydrofuran cycle linked at the C15 (R^*) is inferred from the correlations by NOESY and ROESY (H18–Me37) and by ROESY (H19b–H11) as well as deshielded H11 (δ 3.61).

The partial structure **b** (C19–C27) (Scheme III) was established through analysis of the COSY spectrum[11] and by the correlations observed on the HMBC map between H24/C23, H22/C23, H21/C23, H22/C20, H21/C20, H19/C20, H38/C20, and H18/C20. Concerning the carbons C21 (R^*) and C24 (S^*), the stereochemistry was determined by the correlations suggested by ROESY H19a/Me(38), H22/H11, H24/Me(38), H21/H11, and H21/H18 (Scheme IV). The lack of cross-peak correlation in the COSY allows us to consider the coupling constant of ${}^3J_{18,19}$ to be near 0, suggesting that the dihedral angle between H18 and H19a is near 90°. The large downfield chemical shift of H18 (δ 4.39) is consistent both with the occurrence of the carbonyl group at C20 and the tetrahydrofuran ring oxygen atom proximity.

As for the partial structure **c** (Scheme V), the interpretation of the COSY spectrum allows to link Me(34) to H2 ($J_{2,34}$ = 0.9 Hz) by a long-range coupling and in the case of lituarine A, H4 is coupled with Me(35) and H5. The three quaternary carbons (C1, C3, and C4) were located on the HMBC spectrum (Scheme V). The carbonyl C1 (δ 165.3) exhibits correlations with H2 and H24. The quaternary ethylenic C3 (δ 162.8) correlates by HMBC with H2, Me(34), and Me(35) and the quaternary carbon C4 (δ 77.9) shows cross-peaks with H2, Me(34), and Me(35) too. The ^{13}C chemical shift values of C2 (δ 114.1) and C3 (δ 162.8) are also consistent with our partial structural assignments.[12-14] The *E* configuration of the $\Delta^{2(3)}$ double

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[7] The ^{1}H–^{1}H COSY spectrum of **3** (a) clearly showed the following connectivities (H–H): 8–9, 9–10, 10–11, 13–14, Me(37)–16, 16–17, 17–18, 18–19.

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[11] The ^{1}H–^{1}H COSY spectrum of **3** (b) clearly showed the following connectivities (H–H): Me(38)–21, 21–22, 24–25, 25–26, 26–27.

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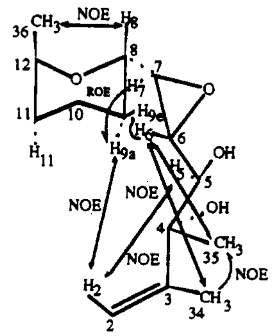
Table I. ^{1}H and ^{13}C NMR Data for Lituarines A, B, and C[a]

	$\delta(^{13}C)$				$\delta(^{1}H)$ (J(Hz))		
no.	1	2	3		1	2	3
1	164.66	165.01	165.35	s[b]			
2	116.12	115.21	114.19	d	5.85	6.25	6.22 (d) ($J_{2,34}$ = 0.9)
3	162.85	161.09	162.84	s			
4	40.10[c]	76.88	77.98	s	2.57		
5	35.91[c]	77.17	74.93	d	1.99–1.16	4.59	3.03 (d) ($J_{5,6}$ = 7.5)
6	52.82	53.20	56.28	d	2.7	3.01	2.78 (dd) ($J_{6,7}$ = 2.2)
7	60.24	58.46	58.28	d	2.42	2.66	2.5 (dd) ($J_{7,8}$ = 6.7)
8	72.22	72.24	72.72	d	3.06	3.11	3.06 (ddd) ($J_{8,9e}$ = 2.5; $J_{8,9a}$ = 11.8)
9	34.46	34.37	34.50	t	1.53–1.67	1.47–1.72	1.5 (9a)–1.6 (9e) (m)
10	23.36	23.23	23.30	t	1.32	1.32	1.36 (m)
11	70.56	70.53	70.66	d	3.6	3.6	3.62 (dd) ($J_{11,10a}$ = 11.8; $J_{11,10e}$ = 4.3)
12	71.53	71.67	71.60	s			
13	28.21	28.23	28.25	t	1.58–1.68	1.61–1.72	1.58–1.71 (m)
14	27.66	27.60	27.71	t	1.56	1.49	1.48–1.62 (m)
15	107.40	107.42	107.43	s			
16	41.73	41.65	41.67	d	1.96	1.94	1.94 (m) ($J_{16,37}$ = 7.0)
17	36.83	36.93	37.00	t	1.49–1.84	1.53–1.85	1.55–1.85 (m) ($J_{17b,16}$ = 0.0)
18	73.58	73.61	73.63	d	4.38	4.35	4.39 (q) ($J_{18,19a}$ = 0.0; $J_{18,17a}$ = $J_{18,17b}$ = 7.8)
19	45.55	45.59	45.84	t	2.28–2.46	2.33–2.42	2.32 (19b) (dd)–2.48 (19a) (d) ($J_{19a,19b}$ = 17.1; $J_{19b,18}$ = 9.6)
20	210.67	210.75	210.8	s			
21	41.68	41.65	41.60	d	2.62	2.68	2.70 (m) ($J_{21,38}$ = 7.9)
22	41.26	41.38	41.48	t	2.90	2.94	2.94 (dd) (dd) (J_{ab} = 16.2; $J_{22,21}$ = 4.0–7.4)
23	204.15	204.11	204.30	s			
24	77.13	77.23	77.06	d	5.22	5.26	5.22 (t) ($J_{24,25}$ = 5.2)
25	33.28	33.36	33.35	t	2.64–2.75	2.61–2.75	2.6–2.75 (m)
26	124.62	124.69	124.77	d	5.50	5.47	5.50 (m) ($J_{26,25}$ = 8.6–6.3)
27	127.85	127.82	127.81	d	6.75	6.74	6.73 (dd) ($J_{27,28}$ = 11.8; $J_{26,27}$ = 14.9)
28	108.02	108.05	108.09	d	5.18	5.16	5.18 (dd) ($J_{28,29}$ = 9.5)
29	121.51	121.50	121.47	d	6.50	6.52	6.50 (t) ($J_{29,NH}$ = 9.7)
30	170.02	169.80	170.04	s			
31	36.83	36.83	36.83	t	2.23	2.23	2.22 (t) ($J_{31,32}$ = 7.2)
32	18.33	18.31	18.31	t	1.53	1.55	1.54 (m)
33	13.47	13.38	13.41	q	0.89	0.89	0.88 (t) ($J_{32,33}$ = 7.4)
34	13.07	15.52	15.73	q	1.97	2.05	1.98 (d) ($J_{34,2}$ = 0.9)
35	20.02	23.51	24.26	q	1.01	1.13	1.22 (s)
36	13.40	13.47	13.46	q	1.02	1.02	1.02 (s)
37	15.09	15.04	15.06	q	0.81	0.81	0.82 (d) $J_{37,16}$ = 7.0)
38	16.97	16.93	16.89	q	1.03	1.04	1.04 (d) ($J_{21,38}$ = 7.9)
39		170.02		s			
40		20.49		q		2.13 (s)	
NH					9.65	9.65	9.73 (d) ($J_{NH,29}$ = 9.7)

[a] Recorded in DMSO-d_6. [b] Multiplicity of carbons was obtained in a DEPT experiment. Carbons bearing hydrogen atoms are edited as follows: CH and CH_3 (positive), CH_2 (negative), and quaternary carbons are erased. [c] In compound 1 according to R_1 and R_2 and C4 and C5 are respectively as d and t.

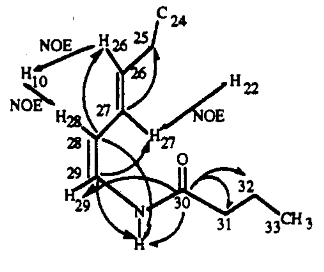
Scheme VI



NOESY Partial Structure d

bond was suggested by the upfield chemical shift value of (δ 15.7) of Me(34).[12,13,15–17] The observed transannular NOE effect between H2 and H9a locates H2 inside the macrocycle.

Scheme VII



HMBC/NOESY Partial Structure e

The partial structure **d**, C4 to C9, was established through the COSY[18] of **3** and confirmed by the C–H long-range (HMBC) correlations (Table I). The trans configuration of the 6,7 oxirane ring was suggested by the coupling constant ($J_{6,7}$ = 2.2 Hz). The NOESY spectrum reveals the location of H6 on the same side of the macrocycle as the 34 and 35 methyl groups (cross-peaks H6 and Me(34); H6 and Me(35)). The relative stereochemistry of C6 (S^*) and C7 (S^*) was established by the ROESY spectrum (cross-peaks H6 and H9e; H7 and H9a) (Scheme

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(18) The ^{1}H–^{1}H COSY spectrum of **3** (d) clearly showed the following connectivities (H–H): 5–6, 6–7, 7–8, 8–9.

Table II. Long-Range Connectivities Observed in the HMBC and NOESY Experiments for Lituarine C

no.	HMBC	NOESY
1	H2, H24	
2	H34	H24, H5, H6, H7, H9, H35
3	H2, H34, H35	
4	H2, H34, H35	
5	H35, H6	H34, H35, H2
6	H5, H7	H9, H34, H35, H22, H10
7	H8, H6	H9, H2, H10
8	H6	H10, H36
9	H11	H7, H6, H11, H2
10	H11	H36, H19, H8, H28, H26, H7
11	H36, H9	H22, H21, H19, H9, H36
12	H11, H36, H13, H14	
13		
14		
15	H18, H11, H37, H16, H13, H14	
16	H17, H37	H17
17	H16, H37	H37, H19
18	H19, H16, H17	H19, H37
19	H17	H17, H38, H10, H11, H22, H18
20	H22, H21, H19, H38, H18	
21	H22, H38	
22	H21	H19, H18, H11, H24, H27
23	H24, H22, H21	
24	H25	H22, H2, H29
25	H27, H26, H24	H27
26	H24, H28, H25	H10
27	H29, H25	H22, H25, NH
28	H27, H29, H26, NH	H10, NH
29	NH, H27, H28	H28
30	NH, H29, H31, H32	
31	H32, H33	NH
32	H31, H33	
33	H32, H31	
34	H2	H35, H6, H5, H2
35	H2	H34, H6, H5, H2
36	H11	H8, H11
37	H17	H17, H18, H13, H14
38	H22, H21	H19, H22
39		
40		
NH		H27, H28, H31

VI). Likely, the H5 proton α to a hydroxyl is located (Scheme VI) inside the macrocycle (cross-peak H5 and H2).

Finally, the structure of the linear C24–C33 chain (partial structure **e**) was established on the one hand by vicinal couplings[19] and on the other hand by HMBC correlations (Scheme VII) with the amide carbonyl C30 (δ 170) (cross-peaks C30/NH, C30/H29, C30/H31, and C30/H32). The E and Z configurations of the $\Delta^{26(27)}$ and $\Delta^{28(29)}$ double bonds were suggested by the coupling constants ($J_{26,27}$ = 15 Hz and $J_{28,29}$ = 9.7 Hz) and confirmed by the ROESY spectrum (cross-peak H26 and H29). The spatial arrangement of this chain, above the macrocycle and on the side of H10, was established through interpretation of the NOESY spectrum (cross-peaks H26/H10, H28/H10, and H27/H22).

The spectral data reported for lituarine C are consistent with our structural assignments. Lituarine A has no hydroxyl group (carbons C4 at δ 38.69 and C5 at δ 35.91), while lituarine B exhibits acetylation on the C5 hydroxyl (C5 at δ 77.17, C39 at δ 170.0, and C40 at δ 20.49).

The CH_2Cl_2 soluble extract proved to inhibit the growth of the following fungi: *Fusarium oxysporum*, *Helminthosporium turscicum*, *Penicillium italicum* and *Phytophtora parasitica*. Further investigations with KB cells showed that purified lituarines were cytotoxic (lituarine A: IC_{50} (3.7–5.0 × 10^{-3} μg/mL; lituarine B: IC_{50} (1–2 × 10^{-3} μg/mL; lituarine C: IC_{50} (5–6 × 10^{-3} μg/mL). Because of these high toxicity, new assays (P-388 lymphocytic leukemia assay) with pure lituarines will be made soon.

Experimental Section

Collection and Isolation. The sea pen *L. australasiae* (Gray, 1970), a pennatulacean octocoral of the Veretillidae family, was collected at night by SCUBA diving near the "Baie de St Vincent" in the western part of the New Caledonian lagoon, on a shallow sandy bottom, and immediately deep-frozen on board. Zoological sampling was made in the meantime.

The freeze-dried animal (fresh weight: 12.5 kg) was first extracted with a mixture of ethanol and water (80/20). The extract was then partitioned into a water-soluble portion and a CH_2Cl_2-soluble one. The latter (96 g) was subjected to silica gel chromatography under vacuum (eluent: CH_2Cl_2 with increasing proportions—from 0 to 3%—of MeOH). The fractions that showed an antifungal activity were joined, then defatted with hexane, and purified by inverse phase column chromatography (silica gel Lichroprep RP 8, 25–40 μm) using a gradient elution of water (from 40 to 20%) in MeOH. Three fractions were obtained. Further purification by the HPLC way was necessary, using a Microporasil column (30 × 0.8). The three fractions were eluted with CH_2Cl_2 and the respective proportions of MeOH (1.0; 1.3; 2.5%). This last step yielded lituarines A (20 mg), B (10 mg), and C (24 mg).

Lituarine A: mp 83–85 °C; UV λ_{max} (MeOH) 270 (ϵ 16 857) nm; IR (film) 3390, 3330, 3050, 2960, 2920, 2860, 1740–1670, 1650, 1620, 1500, 1560, 1380, 1270, 980, 740, and 700 cm^{-1}; FABMS (positive ion, glycerol/trichloroacetic acid matrix) m/z 670 $(M + H)^+$. Anal. Calcd for $C_{38}H_{55}O_9N$: C, 68.13; H, 8.27; N, 2.09. Found: C, 68.32; H, 8.05; N, 2.06. For ^1H and ^{13}C NMR, see Table I.

Lituarine B: mp 126–129 °C; UV λ_{max} (MeOH) 269.5 (ϵ 26 604) nm; IR (film) 3450, 3350, 3060, 2970, 2940, 2880, 1750–1670, 1650, 1620, 1500, 1460, 1370, 1235, 910, 730, and 700 cm^{-1}; FABMS (positive ion, glycerol/trichloroacetic acid matrix) m/z 744 $(M + H)^+$, 726 $(M - H_2O + H)^+$, and 702 $(M - COCH_3 + 2H)^+$. Anal. Calcd for $C_{40}H_{57}O_{12}N$: C, 64.58; H, 7.72; N, 1.88. Found: C, 64.25; H, 7.53; N, 1.82. For ^1H and ^{13}C NMR, see Table I.

Lituarine C: mp 153–157 °C; UV λ_{max} (MeOH) 269 (ϵ 22 419) nm; IR (KBr) 3440, 3350, 3060, 2970, 2940, 2880, 1740–1670, 1650, 1620, 1505, 1460, 1380, 1260, 1210, 1160, 1090, 980, 770, and 660 cm^{-1}; FABMS (positive ion, nitrobenzyl alcohol, CH_3OH matrix) m/z 702 $(M + H)^+$; FABMS (positive ion, nitrobenzyl alcohol, CD_3OD matrix) m/z 704 $(M + 2 + H)^+$. Anal. Calcd for $C_{38}H_{55}O_{11}N$: C, 65.02; H, 7.89; O, 25.07. Found: C, 65.28; H, 7.66; O, 25.05. For ^1H and ^{13}C NMR, see Tables I and II.

Acknowledgment. We thank Marie-José D'hondt (Museum National Histoire Naturelle) for the taxonomic study of *L. australasiae*, Georges Bargibant and Jean-Louis Menou for collection assistance, and Professor Jean-François Verbist (Faculté de Pharmacie de Nantes) for the bioassays, as well as Philippe Sizun and Patrick Berthault (C.E.N. Saclay) for their NMR collaboration.

Supplementary Material Available: FABMS, ^1H NMR, ^{13}C NMR, DEPT(135), ^1H–^1H COSY, ^1H–^{13}C HMQC spectra of lituarines A, B, and C and ROESY (350 ms), NOESY, ^1H–^{13}C HMBC spectra of lituarine C (30 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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[19] The ^1H–^1H COSY spectrum of **3** (e) clearly showed the following connectivities (H–H): 24–25, 25–26, 26–27, 27–28, 28–29, 29–NH, 31–32, 32–33.