Callipeltoside A: A Cytotoxic Aminodeoxy Sugar-Containing Macrolide of a New Type from the Marine Lithistida Sponge *Callipelta* sp.

Angela Zampella,[†] M. Valeria D'Auria,[†] Luigi Minale,*[,†] Cécile Debitus,[‡] and Christos Roussakis[§]

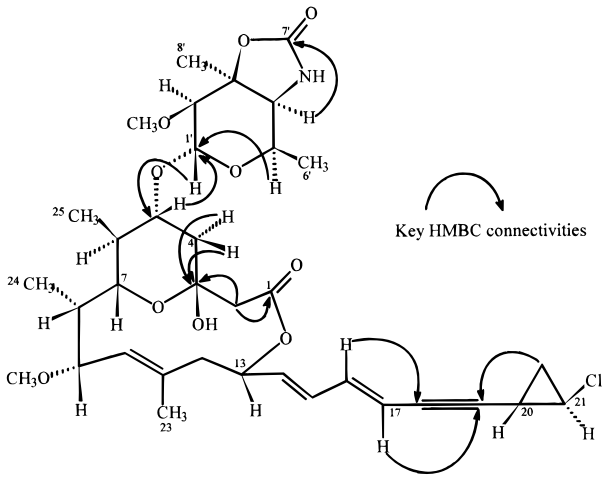
Contribution from the Dipartimento di Chimica delle Sostanze Naturali, Universita' degli Studi di Napoli Federico II, via D. Montesano 49, 80131 Napoli, Italy, ORSTOM, Centre de Nouméa New Caledonia, and Institut des Substances et Organismes de la Mer (ISOMER), Facultè de Pharmacie, University of Nantes, 1-Rue Gaston Veil, 44035 Nantes Cedex 01, France

Received June 21, 1996[⊗]

Abstract: A cytotoxic glycoside macrolide, callipeltoside A, has been isolated from the marine lithistid sponge *Callipelta* sp., collected off New Caledonia. Structural assigment was accomplished through extensive 2D NMR spectroscopy. The complete relative stereochemistry is proposed from the analysis of ROESY and NOE difference experiments. Callipeltoside A (**1**) represents the first member of a new class of marine-derived macrolides, containing unusual structural features including a 4-amino-4,6-dideoxy-2-*O*,3-*C*-dimethyl-α-talopyranosyl-3,4-urethane unit.

Introduction

In our search for new antitumor and antiviral agents from marine sponges, an extract from the shallow water lithistid sponge, *Callipelta* sp., collected in the waters off the east coast of New Caledonia, was found to inhibit *in vitro* proliferation of KB and P388 cells as well to protect cells infected with the HIV virus. We isolated from the dichloromethane−methanol extract the major metabolites responsible for the biological activity, named callipeltins A−C, and assigned a cyclodepsipeptidal structure to A[1] and B,[2] callipeltin C being the acyclic A.[2] Further investigations on the dichloromethane extract from several collections of this marine sponge (2.5 kg freeze-dried in total) have led to the isolation of a new cytotoxic component (3.5 mg yield; 1.4×10^{-4}%, dry weight), which we have named callipeltoside A (**1**). Reported herein are the isolation, structure elucidation, and biological data.



Callipeltoside A (1)

Results and Discussion

Sponge specimens collected several times in 1992−1994 off the east coast of New Caledonia at a depth of 5−10 m were freeze-dried (2.5 kg in total) and sequentially extracted with *n*-hexane, dichloromethane, and 8:2 dichloromethane:methanol. The CH_2Cl_2 fraction was separated by chromatography over silica gel [$CHCl_3$:MeOH (99:1)] and HPLC [reverse phase C-18, H_2O:MeOH (2:8)] to afford callipeltoside A (**1**; 3.5 mg).

Mass spectral and NMR data were consistent with the molecular composition $C_{35}H_{48}O_{10}NCl$; the FABMS gave pseudomolecular ions at *m/z* 700−702 [$(M + Na)^+$, 100, 36] shifted to 716−718 when KCl was added to the glycerol−tioglycerol matrix. The ^{13}C, DEPT (polarization transfer delay adjusted to an average of coupling of 135 Hz), and HMQC NMR spectra in methanol-d_4 indicated 35 distinct resonances, which included two carbonyls, six olefinic carbons (five CH and one quaternary), two internal acetylenic carbons, two oxygenated quaternary carbons, 10 methines [of which seven were oxygenated and one was attached to nitrogen (δ 3.48/62.7)], three methylenes, and seven methyls [of which two were methoxyls (δ 3.63/62.0, δ 3.25/55.4) and one was vinylic (δ 1.78/16.3)]. A DEPT experiment made by using polarization transfer delay adjusted to a CH coupling of 190 Hz revealed one more methylene at δ 19.3 (δ 1.31, m) and two more methines at δ 34.7 (δ 1.85, m) and 55.4 (δ 3.28, m), consistent with the presence of a chlorinated cyclopropane ring, as then confirmed by $^1H-^1H$ COSY. Two deuterium-exchangeable protons were observed at δ 9.16 (s) and 5.98 (br s) (pyridine-d_5). These data accounted for a molecular formula of $C_{35}H_{48}O_{10}NCl$, which requires 12 degrees of unsaturation. Two degrees of unsaturation are accounted for in the presence of two carbonyl carbons. One cyclopropane ring and six olefinic and two acetylenic carbons account for an additional six degrees of unsaturation. Therefore, based on the molecular formula, callipeltoside A (**1**) must contain four more rings. The UV spectrum contained absorptions at λ 250 (shoulder), 272 (ϵ 6727.2), and 286 (shoulder) nm, indicative of a conjugation. Structural elucidation of

* To whom correspondence should be addressed.
[†] University of Naples Federico II.
[‡] ORSTOM.
[§] University of Nantes.
[⊗] Abstract published in *Advance ACS Abstracts,* October 15, 1996.
(1) Zampella, A.; D'Auria, M. V.; Gomez Paloma, L.; Casapullo, A.; Minale, L.; Debitus, C.; Henin, Y. *J. Am. Chem. Soc.* **1996**, *118*, 6202−6209.
(2) D'Auria, M. V.; Zampella, A.; Gomez Paloma, L.; Minale, L.; Debitus, C.; Roussakis, C.; Le Bert, V. *Tetrahedron* **1996**, *52*, 9589−9596.

S0002-7863(96)02100-2 CCC: $12.00

Table 1. ^{1}H and ^{13}C NMR Chemical Shifts and HMBC (Methanol-d_4) and ROESY (Pyridine-d_5) Correlations of Callipeltoside A (**1**) (500 MHz)

	methanol-d_4			pyridine-d_5	
position	H[a] (ppm)	C (ppm)	HMBC[b]	H (ppm)	ROESY[c]
1		172.4			
2	2.56, d (12.7); 2.50, d (12.7)	46.0	C1, C3	2.67, d (12.7); 2.58, d (12.7)	1.57, 5.98
3		97.0			
4	2.25, overlapped; 1.43, t (11.7)	44.5	C3, C5, C6	2.44, dd (12.0, 4.4); 1.57, dt (11.7, 1.7)	5.98, 3.94, 5.02, 0.94
5	3.76, ddd (11.7, 5.9, 4.9)	78.7	C1′, C25	3.94, ddd, overlapped	0.94, 5.02, 5.98
6	1.58, m	39.8		1.64, m	
7	3.68, dd (10.5, 2.4)	76.4	C24	3.81, dd (10.5, 1.5)	0.94, 2.33, 5.98, 3.90
8	2.27, m	38.2	C9, C6	2.33, m	3.90, 0.94
9	3.91, dd (9.5, 2.2)	81.4	C24	3.90, dd (9.8, 2.3)	3.15, 1.70
10	5.30, br dd (9.5)	128.3	C9, C12, C23	5.41, br dd (9.3)	1.10, 2.33
11		134.1			
12	2.37, dd; 2.31, dd, overlapped	47.8	C10, C11, C13, C23	2.33, dd; 2.25, dd, overlapped	5.88
13	5.86, overlapped	72.7		5.88, m	1.70
14	5.87, overlapped	134.2	C13, C16	5.80, dd (15.1, 6.3)	1.70, 6.54
15	6.37, dd (14.2, 10.5)	132.0	C13, C17	6.32, dd (15.1, 10.8)	5.72
16	6.53, dd (15.3, 10.5)	141.5	C14, C18	6.54, dd (15.3, 10.8)	
17	5.68, dd (15.3, 1.7)	113.4	C15, C19	5.72, dd (15.3, 1.0)	
18		78.3			
19		92.4			
20	1.85, m, overlapped	34.7		1.87, m	
21	3.28, m	55.4		1.14, m	
22	1.31, m	19.3	C19	3.31, m	
23	1.78, s	16.3	C10, C11, C12	1.70, s	
24	0.99, d (6.9)	6.8	C7, C8, C9	1.10, d (7.3)	
25	1.03, d (6.4)	12.8	C5, C6, C7	0.94, d (6.4)	3.52
OCH_3	3.25, s	55.4	C9	3.15, s	
OH				5.98, br s (1.7)	
1′	4.74, d (6.1)	103.6	C5, C5′	5.02, d (6.2)	3.52, 0.94
2′	3.42, d (6.1)	83.2	C1′, OCH_3	3.48, d (6.2)	1.48, 3.96
3′		83.8			
4′	3.48, d (1.7)	62.7	C5′, C7′, C8′	3.42, d (1.4)	1.48, 3.96, 9.16
5′	3.99, dq (6.5, 1.7)	65.3	C1′, C4′, C6′	3.96, dq, overlapped	
6′	1.12, d (6.5)	15.9	C4′, C5′	1.08, d (6.6)	9.16
7′		161.2			
8′	1.54, s	23.0	C2′, C3′, C4′	1.48, s	
OCH_3	3.63, s	62.0	C2′	3.52, s	
NH				9.16, s	

[a] Coupling constants are in parentheses and given in Hz. [b] HMBC optimized for $J_{2,3} = 6$ Hz. [c] ROESY mixing time $t_m = 500$ ms.

callipeltoside A (**1**) required 500 MHz 2D NMR analysis ($^{1}H-^{1}H$ COSY, HMQC,[3] HMBC,[4] ROESY[5]) and NOE difference experiments employing methanol-d_4 and pyridine-d_5 as solvents. The assignments recorded in Table 1 are illustrative but require a supporting discussion, here reported.

Macrolide Portion. Connectivities from C4 to C17 were inferred from the COSY cross-peaks, *viz*., H4a/H5, H4b/H5, H5/H6, H6/25Me, H6/H7, H7/H8, H8/24Me, H8/H9, and H9/H10. Allylic couplings between H10 and 23Me at δ 1.78 and between H10 and H12 at δ 2.31 led connectivity from C10 to C12. The *E* geometry of the C10−C11 double bond was delucidated on the basis of the ^{13}C NMR chemical shift of the 23Me signal at δ 16.3 and confirmed by observation of ROESY correlations from H10 to $H_2$12 and from 23Me to H9. The COSY spectrum showed both the signals at δ 2.31 and 2.37 ($H_2$12) to be coupled to the oxymethine at δ 5.86 (H13), which in turn was coupled to the *E* olefinic protons ($J_{14,15} = 14.2$ Hz) at δ 5.87 (H14) and 6.37 (H15); these latter were coupled with the *E* olefinic protons ($J_{16,17} = 15.3$ Hz) at δ 6.53 (H16) and 5.68 (H17), which together gave rise to a diene chromophore. The chemical shift of H13 (δ 5.86) suggested that C13 was participating in an ester linkage. The chemical shifts at δ 3.76, 3.68, and 3.91, assigned to H5, H7, and H9, respectively, indicated that C5, C7, and C9 were oxygenated, and a HMBC cross-peak between the methoxyl singlet signal at δ 3.25 and the carbon signal at δ 81.4 assigned to C9 placed a methoxyl group there. Connectivities from C17 to C19 were revealed on the basis of HMBC correlations H16−C18 (δ 78.3) and H17−C19 (δ 92.4). In the COSY spectrum the olefinic proton H17 (δ 5.68) showed fine long range coupling ($J = 1.7$ Hz) to the cyclopropane multiplet H20 (δ 1.85), thus allowing placement of the chlorinated cyclopropane at C19 as a terminal grouping. This connection was supported by HMBC correlations from $H_2$22 to C19 (δ 92.4). Multiple COSY correlations between H20/$H_2$22, H20/H21, and H21/$H_2$22 confirmed the presence of a cyclopropane substituted by the chlorine atom as implied by the proton signal of H21 downfield shifted to δ 3.28 and the chemical shift for C21 at 55.4 ppm. The stereochemistry was determined from the proton-coupling constant ($J_{20,21} = 3.1$ Hz), measured after removal of the splitting due to $J_{21,22}$ by spin decoupling, associated with a *trans* disubstituted cyclopropane.[6] HMBC data connected the C4 methylene to the hemiketal carbon at δ 97.0 ppm (C3), which in turn was correlated with the isolated C2 methylene protons (δ 2.50, d; 2.56, d; $J = 12.7$ Hz), which were indicated to be vicinal to a carbonyl carbon as judged by the chemical shifts, and confirmed by HMBC correlation from $H_2$2 to the carbonyl signal at δ 172.4 ppm. Thus, the connectivity from C4 to C2-CO via a C3 hemiketal was elucidated. The location of the hemiketal ring between C3 and C7 was determined on the basis of a ROESY

(3) Bax, A.; Griffey, R. H.; Hawkins, B. L. *J. Am. Chem. Soc*. **1983**, *105*, 7188−7190.
(4) Bax, A.; Summers, M. F. *J. Am. Chem. Soc*. **1986**, *108*, 2093−2094.
(5) Bax, A.; Davis, A. G. *J. Magn. Reson.* **1985**, *66*, 207−213.
(6) Breitmaier, E. *Structure Elucidation by NMR in Organic Chemistry*; John Wiley & Sons: Chichester, 1993; pp 42−46.

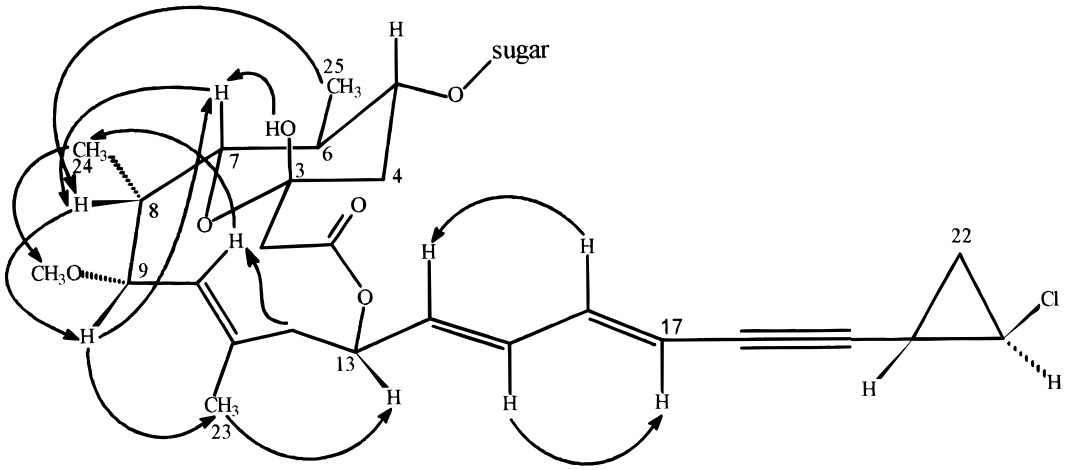


Figure 1. NOE enhancements observed in NOE difference experiments of callipeltoside A (**1**).

correlation between H7 and the exchangeable proton at δ 5.98 (pyridine-d_5, 3-OH). Large vicinal couplings ($J_{6,7}$ = 10.5 Hz, $J_{5,6}$ = 5.9 Hz, $J_{5,4a}$ = 11.7 Hz) suggested the presence of a six-membered ring with H5, H6, and H7 axially disposed. A ROESY correlation between H5 and 3-OH confirmed their axial positions, indicating the chair conformation depicted in Figure 1. Finally, a lactone was placed between C1 and the oxygen on C13 mainly on the basis of the chemical shift of H13 at δ 5.86 (δ 72.7).

The study of the relative stereochemistry of the macrolide portion of the molecule was continued through analysis of ROESY data (in pyridine-d_5, Table 1) and NOE difference experiments in methanol-d_4 (Figure 1). ROESY correlations H7/H8, H7/H9, H8/25Me, and 9-OCH_3/24Me revealed the relative stereochemistry at C8 and C9 with H8 and H9 disposed on the same face of H7. Further, ROESY correlations H8/25Me, H10/24Me, H10/H12, and H9/23Me placed a constraint on the C8−C12 portion; thus the observed ROESY correlation between H13 and the vinylic Me at C23 allowed us to correlate the configuration of C13 to that of C9 and C8. Analysis of the Dreiding molecular models revealed that of the two possible relative stereochemistries for C13, only that depicted in Figure 1 would account for the observed ROESY correlations between the olefinic H10 and both 24Me and H12 and between the vinylic 23Me and both H13 and H9. The same results were obtained by employing NOE difference spectroscopy in methanol-d_4 (Figure 1). Irradiation of 25Me at δ 1.03 indicated enhancement of the signals for H8 (δ 2.27) and H6 (δ 1.58), while irradiation of the signal for H9 (δ 3.91) indicated enhancement of the signal for 23Me at δ 1.78. Then, irradiation of the 23-vinyl methyl resonance at δ 1.78 indicated enhancement of the signals for H9 (δ 3.91) and H13 (δ 5.86), while irradiation of the olefinic resonance at δ 5.30 indicated enhancement of the signals for 24Me (δ 0.99) and one of the two methylene hydrogens at C12 (δ 2.31).

Sugar Portion. Further analysis of the NMR data, starting from the proton signal at δ 4.74 (H1′), which was indicated to be hemiacetal from the chemical shift of the attached carbon at δ 103.6 ppm, allowed us to identify one previously unknown 4-amino-4,6-dideoxy-2-*O*,3-*C*-dimethyl-α-pyranosyl-3,4-urethane unit. The COSY spectrum showed connectivities from C1′ to C2′ and from C4′ to C6′. An HMBC cross-peak between H2′ and the carbon signal at δ 62.0 ppm (methoxyl carbon) placed the second methoxyl group at C2′. HMBC correlations from H4′ (CHN δ 3.48/62.7) to C8′ (δ 23.9) and from 8′Me (δ 1.54) to C3′ (δ 83.8) connected C3′ to C4′. ROESY correlations of H2′ to 8′Me and of H4′ to 8′Me connected C2′ to C3′ and revealed the *syn* stereochemistry of H2′/8′Me and H4′/8′Me,

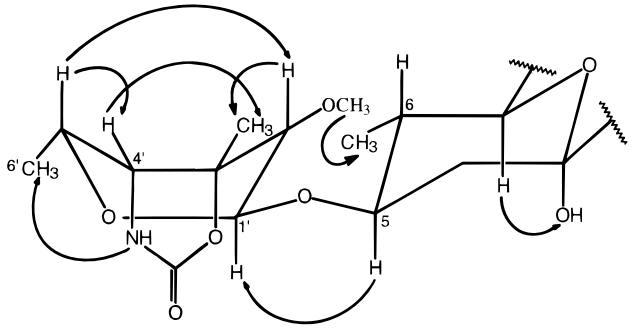


Figure 2. NOE enhancements observed for the sugar portion of callipeltoside A (**1**).

which was confirmed by NOE difference experiments in methanol-d_4 (Figure 2). Thus the connection C2′ to C4′ *via* the nonprotonated C3′ was established. A key HMBC cross-peak between H4′ and the carbonyl carbon signal at δ 161.2 (C7′) allowed the placement of the second carbonyl between the oxygen on C3′ and the nitrogen on C4′ giving rise to a cyclic urethane function. Finally the glycopyranosyl structure was confirmed by observation of HMBC correlation from H1′ to C5′; ROESY correlations between H4′ and H5′ and between 6′Me and NH (δ 9.16, in pyridine-d_5) indicated a *syn* arrangement of H4′ and H5′. The large coupling constant (J = 6.1 Hz) of H1′/H2′ suggested a *trans* arrangement of these two hydrogens. Therefore we conclude the relative stereochemistry of the sugar moiety as the 4-amino-4,6-dideoxy-3-*C*,2-*O*-dimethyl-α-talopyranosyl.

H2′ exhibited ROESY correlation to H5′ indicative for a boat conformation (Figure 2). The connection of the sugar moiety to the C5 of the macrolide through a glycoside linkage was derived by observation of HMBC correlations from H1′ to C5 and from H5 to C1′. Strong NOEs observed between the anomeric proton at δ 4.74 and the macrolide H5 at δ 3.76, as well as between the 2′-OCH_3 signal at δ 3.63 (s) and the doublet of methyl protons at C6 δ 1.03 of the macrolide, put a rotational constraint around the glycoside linkage, allowing us to interrelate the relative stereochemistry of the sugar and the macrolide portion.

Conclusion

Callipeltoside A (**1**) is the first member of an unprecedented class of marine natural products with unusual structural features such as the previously unknown 4-amino-4,6-dideoxy-2-*O*,3-*C*-dimethyl-α-talopyranosyl-3,4-urethane (callipeltose), linked through an α-*O*-glycoside linkage to an hemiketal oxane ring,

Table 2. Effects of Callipeltoside A (**1**) on the NSCLC-N6 Cell Cycle

	cells in G1 phase (%)	cells in S phase (%)	cells in G2/M phase (%)
control	67.9	28.0	4.1
callipeltoside A (**1**) 30 μg/mL	82.5	14.9	2.6
10 μg/mL	77.5	20.8	1.7
5 μg/mL	74.7	22.6	2.7

which is part of a 14-membered macrocycle lactone with a dienyne cyclopropane side chain.

Marine glycoside macrolides, polycavernoside A and its analogs, have recently been described as the toxins responsible for the toxicity resulting from the ingestion of the red alga *Polycavernosa tsudai*.[7] Branched-chain sugars have been reported as constituents of antibiotic macrolides produced by numerous microorganisms. The 6-deoxy-2-*O*,3-*C*-dimethyl-L-talose (vinelose) and the 4,6-dideoxy-3-*C*-methyl-4-(methylamino)-L-mannose (sibirosamine), both somewhat similar to callipeltose, were found to occur as components of two cytidine nucleosides isolated from cultures of *Azobacter vinelandii*[8] and as the carbohydrate portion of sibiromycin, an antitumor antibiotic, isolated from a culture of *Streptosporangium sibiricum*,[9] respectively. This may suggest the microbial origin of callipeltoside A.

Similar to several other marine-derived macrolides, including acutiphicin,[10] cynarchyrolide A,[11] althohyrtin A,[12] aplyronine A,[13] and spongiastatin 1,[14] callipeltoside A is a cytotoxin, even if it has a moderate activity with IC_{50} values against the NSCLC-N6 human bronchopulmunary non-small-cell-lung carcinoma and P388 of 11.26 and 15.26 μg/mL, respectively. Further, cell cycle analysis by flow cytometry assays of the NSCLC-N6 cell line treated with callipeltoside A (**1**) revealed a cell cycle-dependent effect, involving a dependent G1 blockage (Table 2). These results are indicative of a blockage of the NSCLC-N6 cell proliferation *in vitro* at the level of the G1 phase or by enzyme inhibition or inducing terminal cell differentiation. In the latter case callipeltoside A would be an interesting mechanism-based lead. Further biological evaluation of **1** is in progress.

Experimental Section

General Information. For general information, see: Zampella et al.[1]

Isolation. *Callipelta* sp. (Demospongiae, Lithistida, Corallistidae) was collected in 1992 and 1993 in the shallow waters off the east coast of New Caledonia. Taxonomic identification was performed by Professor Claude Lévi, Muséum National d'Histoire Naturelle, Paris, France, and reference specimens are on file (reference 1572) at the ORSTOM Centre of Nouméa. Preliminary tests of bioactivity on polar extracts showed antifungal activity against *Fusarium oxysporum*, *Helminthosporium sativum*, and *Phytophtora hevea*, cytotoxic activity against KB and P388 cells (10 μg/mL, *ca*. 80% inhibition, in both cases), and anti-HIV activity.

The organisms were freeze-dried, and the lyophilized matherial (2.5 kg) was extracted with *n*-hexane and CH_2Cl_2 in a Soxhlet apparatus and then with CH_2Cl_2:MeOH (8:2, 3 × 2 L) at room temperature. The dichloromethane extracts were filtered and concentrated under reduced pressure to give 4.0 g of a brown oil. The crude dichloromethane extract was chromatographated on a silica gel column ($CHCl_3$:MeOH). The fraction eluted with 99:1 $CHCl_3$:MeOH was further purified by HPLC on a μ-Bondapak C-18 column (flow rate 2 mL/min) with MeOH:H_2O (8:2) as eluent to give 3.5 mg of pure **1** (t_R = 6.8 min): $[\alpha]_D = -17.6°$ (*c* 0.04, MeOH); UV (MeOH) λ 250 (ϵ 6727.2), 272 (ϵ 8031.8), 286 nm (ϵ 6272.7); IR (KBr) 1747, 1732, 1651−1578 (br), 1385, 1050 cm^{-1}; ^1H and ^{13}C NMR in Table 1; FABMS *m/z* 700−702 $[(M + Na)^+]$.

Determination of Biological Activity. Cytotoxic Assays. Experiments were performed in 96-well microtiter plates (2×10^5 cells/mL). Cell growth was estimated by a colorimetric assay based on conversion of tetrazolium dye (MTT) to a blue formazan product using live mitochondria.[15] Eight determinations were performed for each concentration. Control growth was estimated for 16 determinations. Optical density at 570 nm corresponding to solubilized formazan was read for each well on a Titertek Multiskan MKII instrument.

Flow Cytometry Assays. For DNA staining, 2×10^5 cells were cultured in 25 mL flasks in the absence and presence of product. DNA staining was carried out using the technique of Vindelov. Solution [0.01 M glycine/NaOH, 9.6×10^{-5} M propidium iodide, 0.1 M Nonidet P-40, 700 IU ribonuclease A/1, 0.3 M NaCl; diluted 1:2 (v/v) in phosphate-buffered saline] was dropped into the flasks, which were then shaken and left in a dark environment at 4 °C for 15 min. The cell suspension thus obtained was filtered on nylon mesh (50 μm) and analyzed. Each histogram was performed with a DNA content of at least 30 000 nuclei on a Becton Dickinson FACScan instrument. To eliminate doublets, nuclei were selected by gating on the cytogram "DNA peak vs DNA area".

Acknowledgment. This contribution is part of the ORSTOM−CNRS SMIB program; we thank EEC for financial support (Contract MAS 3-CT95-0032). Mass spectra were provided by the Servizio di Spettrometria del CNR e dell'Università di Napoli. The staff is acknowledged. The NMR spectra were recorded at CRIAS Centro Interdipartimentale di Analisi Strumentale, Faculty of Pharmacy, University of Naples. We are grateful to Professor Claude Lévi, Muséum National d'Historie Naturelle, Paris, France, for the identification of the sponge.

Supporting Information Available: ^1H and ^{13}C NMR, COSY, HOHAHA, HMQC, HMBC, and ROESY of callipeltoside A (**1**) (9 pages). See any current masthead page for ordering and Internet access instructions.

JA9621004

(7) (a) Youtsu-Yamashita, M.; Haddock, R. L.; Yasumoto, T. *J. Am. Chem. Soc.* **1993**, *115*, 1147−1148. (b) Youtsu-Yamashita, M.; Seki, T.; Paul, V. J.; Naoki, H.; Yasumoto, T. *Tetrahedron Lett.* **1995**, *36*, 5563−5566.

(8) (a) Okudi, S.; Suzuki, N.; Suzuki, S. *J. Biol. Chem.* **1967**, *242*, 958−966. (b) Brimacombe, S. J.; Mahmood, S.; Rollins, A. J. *J. Chem. Soc., Perkin Trans. I* **1975**, 1292−1297.

(9) Parker, K. A.; Babine, R. E. *J. Am. Chem. Soc.* **1982**, *104*, 7330−7331.

(10) Barchi, J. J.; Moore, R. E.; Patterson, G. M. L. *J. Am. Chem. Soc.* **1984**, *106*, 8193−8197.

(11) Fusetani, N.; Shinoda, K.; Matsunaga, S. *J. Am. Chem. Soc.* **1993**, *115*, 3977−3981.

(12) Kobayashi, M.; Aoki, S.; Sakai, H.; Kawazoe, K.; Kihara, N.; Sasaki, T.; Kitagawa, I. *Tetrahedron Lett.* **1993**, *34*, 2795−2798.

(13) Yamada, K.; Ojika, M.; Ishigaki, T.; Yoshida, Y. *J. Am. Chem. Soc.* **1993**, *115*, 11020−11021.

(14) Pettit, G. R.; Zbigniew, A. C.; Cichacz, F. G.; Gao, F.; Herald, C. L.; Boyd, M. R.; Schmidt, J. M.; Hooper, J. N. A. *J. Org. Chem.* **1993**, *58*, 1302−1304.

(15) Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55−68.