6. Leucascandrolide A, a New Type of Macrolide: the First Powerfully Bioactive Metabolite of Calcareous Sponges (*Leucascandra caveolata*, a New Genus from the Coral Sea)

by **Michele D'Ambrosio**[a]), **Antonio Guerriero**[a]), **Cécile Debitus**[b]), and **Francesco Pietra**[a])*

[a]) Istituto di Chimica, Università di Trento, I–38050 Povo-Trento
[b]) ORSTOM, Centre de Nouméa, B.P. A5 Nouméa Cédex, Nouvelle Calédonie

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Leucascandrolide A ((+)-**1**), a doubly O-bridged 18-membered macrolide of a new type, *i.e.*, showing little C_1-branching *vs.* extensive 1,3-dioxygenation and a peculiar side chain, was isolated from a calcareous sponge of a new genus, *Leucascandra caveolata* BOROJEVIC and KLAUTAU from the Coral Sea. Transesterification of (+)-**1** gave the methyl ester **3**, derived from the side chain, and the 5-hydroxy derivative (+)-**2**, derived from the macrolide portion and with the natural configuration at C(5) (axial). *Mosher*'s MTPA esters **4** and **5** obtained from (+)-**2** showed scattered $\Delta\delta = (\delta(S) - \delta(R))$ data. However, inversion of the configuration at C(5) led, *via* ketone (+)-**6**, to the less encumbered 5-equatorial hydroxy derivative (+)-**7**, whose MTPA esters **8** and **9** gave consistent $\Delta\delta$ data, allowing the assignment of the absolute configuration of (+)-**7**, and hence of (+)-**1**. The structural novelty of (+)-**1** and its powerful antifungal and cytotoxic activities are likely to renew interest in calcareous sponges, previously limited to scarcely biologically active 2-aminoimidazoles.

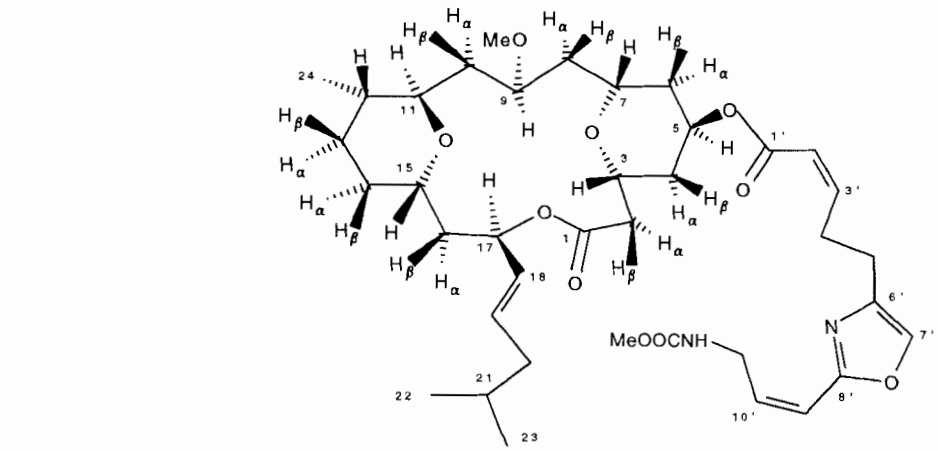
1. Introduction. – Calcareous sponges, being few and of low biomass [1], have allured the organic chemist in search of new molecules less than the myriad of fleshy demosponges. As far as we know, only the calcareous genera *Leucetta* [2], *Clathrina* [3], *Grantia*, *Leuconia*, *Sycon*, and *Ascaltis*[1]) were systematically examined; unusual metabolites were found in sponges of the first two genera only, and limited to characteristic 2-aminoimidazoles that embody one or two benzyl-substituted moieties, or derived groups [2–4], and Zn^{++} complexes from them [2b] [3] [4], all endowed of scarce bioactivity. Specific studies as to fatty-acid composition in calcareous sponges were carried out for *Ascandra* sp., *Clathrina* sp., *Sycon* sp. [5a], *Leucettusa lancifer* [5b], and *Leucosolenia canariensis* [5c].

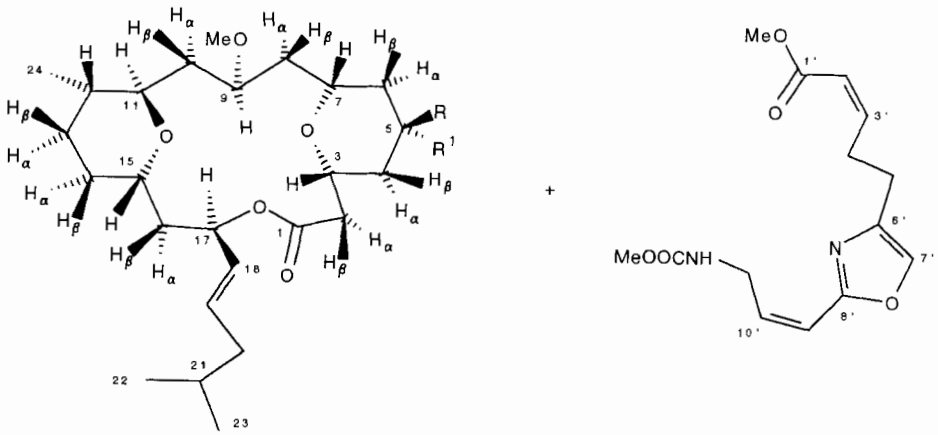
In the search of molecular diversity from biodiversity of calcareous sponges, we finally came to a breakthrough with the new genus *Leucascandra caveolata* BOROJEVIC and KLAUTAU, collected along the east coats of New Caledonia, Coral Sea. This sponge gave the first powerful bioactive metabolite from calcareous sponges, leucascandrolide A ((+)-**1**), which also represents a new type of macrolide.

2. Results and Discussion. – 2.1. *Gross Structure.* The composition $C_{38}H_{56}N_2O_{10}$ for leucascandrolide A ((+)-**1**) was deduced from HR-EI-MS, in agreement with 1D

[1]) In unpublished studies, we previously examined calcareous sponges from the coasts of Brittany (*Grantia compressa*, collections 285M and 375M, as well as *Leuconia* sp., collections 246–248M, 286M, and 386M), Tuscany (*Sycon* sp., collection 27M), and New Caledonia (*Ascaltis grisea*, collection 438M).

Scheme





(+)-**2** R = OH, R' = H
4 R = O[(*S*)-MTPA], R' = H
5 R = O[(*R*)-MTPA], R' = H
(+)-**6** R, R' = O
(+)-**7** R = H, R' = OH
8 R = H, R' = O-(*S*)-MTPA
9 R = H, R' = O-(*R*)-MTPA

b) *c*) *d*) *e*) *f*) *g*)

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a) MeOH, Na_2CO_3, r.t., 2 d, then TLC; 77% yield. *b*) (*R*)-MTPA-Cl, pyridine, r.t., 5 h, then TLC; 83% yield. *c*) Like *b*), using (*S*)-MTPA-Cl; 67% yield. *d*) PCC, CH_2Cl_2, r.t., 2 h, then TLC; 70% yield. *e*) $NaBH_4$ excess, EtOH, r.t., 1 h, then TLC; 86% yield. *f*) (+)-**7**/(+)-**2** 95:5, (*R*)-MTPA-Cl, pyridine, r.t., overnight, then TLC; 80% yield. *g*) Like *f*) with (*S*)-MTPA-Cl; 80% yield.

^{13}C-NMR spectra and DEPT data. A total of 38 ^{13}C resonances showed up for 1 trisubstituted and 3 disubstituted olefinic bonds, 7 *O*-bound CH groups, 2 MeO groups, 12 CH_2 groups, and 1 i-Pr group, besides 4 signals at δ(C) > 158. Four cycles were thus implied.

Because of extensive superimposition of ^{1}H-NMR signals in the 1.2–1.9 ppm zone, any further progress in structural elucidation of (+)-**1** required assigning protons to C-atoms, which was achieved by HMQC experiments (*Table 1*). This allowed us to analyze DQ-COSY maps, thus establishing the set of connectivities from C(2) to C(23)[2]). These attributions were confirmed by HMBC data, allowing us, from data in *Table 1*, to establish the sequences C(1)–C(2), C(1)–O–C(17), C(3)–O–C(7), and C(11)–O–C(15), as well as to assign the δ(C) at 56.61 ppm to MeO at C(9).

Table 1. *^{13}C-NMR Data* (C_5D_5N) *of Leucascandrolide A* ((+)-**1**), *its Hydroxy-Substituted Fragment* (+)-**2**, *the Epimer* (+)-**7**, *and the Oxidized Form* (+)-**6**. Arbitrary numbering.

	(+)-**1**[a])	(+)-**2**	(+)-**6**	(+)-**7**	^{1}H,^{13}C Long-range correlations for (+)-**1**
C(1)	169.75 (*s*)	170.28 (*s*)	169.27 (*s*)	169.98 (*s*)	2 H–C(2), H–C(17)
C(2)	43.54 (*t*)	43.92 (*t*)	43.59 (*t*)	43.56 (*t*)	
C(3)	70.14 (*d*)	69.85 (*d*)	73.73 (*d*)	72.90 (*d*)	H_α–C(2), H–C(7)
C(4)	35.66 (*t*)	39.63 (*t*)[b])	47.34 (*t*)	42.06 (*t*)	H_α–C(2)
C(5)	67.77 (*d*)	63.73 (*d*)	205.24 (*s*)	67.30 (*d*)	H_β–C(6)
C(6)	35.86 (*t*)	39.88 (*t*)[b])	47.94 (*t*)	42.42 (*t*)	
C(7)	70.21 (*d*)	69.66 (*d*)	74.19 (*d*)	73.39 (*d*)	H_α–C(8)
C(8)	39.50 (*t*)	39.47 (*t*)[b])	39.76 (*t*)	39.67 (*t*)	
C(9)	73.54 (*d*)	73.88 (*d*)	73.87 (*d*)	74.02 (*d*)	H_α–C(8), H_β–C(8), MeO–C(9)
*Me*O–C(9)	56.61 (*q*)	56.65 (*q*)	56.76 (*q*)	56.66 (*q*)	H–C(9)
C(10)	35.58 (*t*)	35.82 (*t*)	35.79 (*t*)	35.76 (*t*)	H–C(11)
C(11)	73.75 (*d*)	73.82 (*d*)	73.77 (*d*)	73.84 (*d*)	H_β–C(10), H–C(9), Me–C(12)
C(12)	31.34 (*d*)	31.38 (*d*)	31.38 (*d*)	31.47 (*d*)	Me–C(12), H–C(11)
Me–C(12)	18.38 (*q*)	18.42 (*q*)	18.42 (*q*)	18.44 (*q*)	H–C(11)
C(13)	24.31 (*t*)	24.22 (*t*)	24.28 (*t*)	24.34 (*t*)	Me–C(12), H–C(11)
C(14)	27.38 (*t*)	27.35 (*t*)	27.47 (*t*)	27.52 (*t*)	
C(15)	63.11 (*d*)	63.11 (*d*)	63.12 (*d*)	63.11 (*d*)	H_α–C(16), H_α–C(14), H–C(11), H–C(17)
C(16)	43.22 (*t*)	43.36 (*t*)	43.28 (*t*)	43.42 (*t*)	H–C(17), H–C(18)
C(17)	70.99 (*d*)	70.89 (*d*)	71.32 (*d*)	70.97 (*d*)	H_α–C(2), H_β–C(16)
C(18)	131.28 (*d*)	131.49 (*d*)	131.19 (*d*)	131.44 (*d*)	2 H–C(20)
C(19)	132.03 (*d*)	131.93 (*d*)	132.31 (*d*)	132.01 (*d*)	H–C(17), H–C(21)
C(20)	41.66 (*t*)	41.71 (*t*)	41.71 (*t*)	41.72 (*t*)	H–C(18), H–C(19), H–C(21), 3 H–C(22)
C(21)	28.27 (*d*)	28.31 (*d*)	28.30 (*d*)	28.31 (*d*)	2 H–C(20), 3 H–C(22)
C(22)	22.24 (*q*)	22.28 (*q*)	22.28 (*q*)	22.28 (*q*)	2 H–C(20), H–C(21)
C(23)	22.21 (*q*)	22.24 (*q*)	22.24 (*q*)	22.24 (*q*)	2 H–C(20), H–C(21)

[a]) Chemical shifts, multiplicity, and ^{1}H,^{13}C-long-range correlations for C-atoms of the oxazol-containing side chain of (+)-**1**: 165.44 (*s*, C(1′), H–C(2′) and H–C(3′)); 120.98 (*d*, C(2′), 2 H–C(4′)); 149.28 (*d*, C(3′), 2 H–C(4′) and 2 H–C(5′)); 28.12 (*t*, C(4′), H–C(2′), H–C(3′), and 2 H–C(5′)); 25.91 (*t*, C(5′), H–C(3′) and 2 H–C(4′)); 141.51 (*s*, C(6′), H–C(7′), 2 H–C(5′), and 2 H–C(4′)); 134.43 (*d*, C(7′), 2 H–C(5′)); 160.45 (*s*, C(8′), H–C(7′), H–C(10′), and 2 H–C(11′)); 115.36 (*d*, C(9′), 2 H–C(11′)); 138.65 (*d*, C(10′), H–C(9′) and 2 H–C(11′)); 40.72 (*t*, C(11′), H–C(9′)); 157.91 (*s*, NH*C*OOMe, 2 H–C(11′) and NHCOO*Me*); 51.80 (*q*, NHCOO*Me*).

[b]) These resonances can be interchanged.

[2]) Arbitrary numbering.

The position of the side-chain ester group was shown to be C(5) from typically deshielded ^{1}H resonances. This suggested that the remaining C-, H-, N-, and O-atoms must reside in the (monocyclic) ester side chain, for which DQ-COSY maps established the connectivities from C(2′) to C(7′) and from C(9′) to C(11′)–NH, which could be further extended to C(8′) to C(11′)–NHCOOMe from HMBC data (*Table 1*). The latter also confirmed C(6′)–C(7′) bonding and established the C(2′)–C(1′) and C(8′)–C(7′) bonds. On account of the remaining N- and O-atoms, and NMR signals fitting for a 2,4-disubstituted oxazole [6], the entire side chain was thus elucidated.

2.2. *Relative Configuration.* A clear-cut pattern of *J* couplings emerged from the analysis of spectra of leucascandrolide A, implying a rigid structure. The assignments as shown in structure (+)-**1** were based on the following features: H–C(3), H–C(7), and H–C(15) on two large *trans*-diaxial couplings in each case, H–C(5) on small diequatorial and equatorial-axial couplings, H–C(11) on a small diequatorial coupling with H–C(12) and a large *trans*-antiperiplanar coupling with H_β–C(10), and, finally, H_α–C(10) on a large *trans*-antiperiplanar coupling with H–C(9). This established the relative configurations within both tetrahydropyran moieties, which could be interrelated as shown in (+)-**1** from an intense ROESY map between H–C(7) and H_β–C(10); further support to this assignment will emerge below, following the assignment of the absolute configuration at C(5).

A ROESY map between the protons at C(9) and C(17) supported their *endo*-position. (*Z*)-Configuration at C(2′)=C(3′) and C(9′)=C(10′) was deduced from typical *J* values (11.0 and 12.0 Hz, resp.).

2.3. *Absolute Configuration.* On the presumption that the macrolide moiety of leucascandrolide A ((+)-**1**) could be easily freed, its chiral centre C(5) looked promising to establish the absolute configuration using *Mosher*'s NMR methodology [7a]. In fact, transesterification of (+)-**1** in MeOH in the presence of Na_2CO_3 gave methyl ester **3** derived from the estereal side chain and the hydroxy derivative (+)-**2** with the natural configuration at C(5) (see *Scheme*). From the latter, the diastereoisomeric MTPA esters **4** and **5** could be obtained by standard procedures (MTPA = α-methoxy-α-(trifluoromethyl)phenylacetic acid = 3,3,3-trifluoro-2-methoxy-2-phenylpropanoic acid), revealing negative values for $\Delta\delta = \delta(S) - \delta(R)$ along the lactone-bond side and positive values along the opposite side, with the exception, however, of the equatorial H-atoms at C(4) and C(6) (*Table 2*). Such a deviation from the typical *Mosher*'s trend [7a], which was not too surprising for a sterically encumbered axial OH group [7b], precluded confident application of *Mosher*'s NMR method; failure of the method was attributed to a departure – imposed by repulsive interactions among H_{ax}–C(3), H_{ax}–C(7), and the MTPA moiety – from the ideal *Mosher*'s conformation, *i.e.*, the conformation with H–C(5) in the same plane as the MTPA C=O group and the CF_3 group. The obvious remedy was then to invert the configuration at C(5) to have a less bulky environment. This was achieved by smooth oxidation of (+)-**2** with pyridinium chlorochromate (PCC) in CH_2Cl_2 to the ketone (+)-**6** (70% yield), followed by reduction with $NaBH_4$ in EtOH to give the inverted alcohol (+)-**7** in 86% yield, accompanied by a mere 5% of (+)-**2**. Esterification of (+)-**7** with MTPA-Cl afforded the diastereoisomeric MTPA esters **8** and **9**, where the trend of $\Delta\delta = \delta(S) - \delta(R)$ proved opposite to that found for the epimeric **4** and **5**, without exceptions for all the detectable signals (*Table 2*). This allowed us to assign with confidence the (*S*)-configuration to C(5) of (+)-**7**, and thus (5*R*)-configuration to leucascandrolide A

Table 2. *^{1}H-NMR Data* ($CDCl_3$) *for Leucascandrolide A* ((+)-**1**), *Its Hydroxy-Substituted Fragment* (+)-**2** *and Epimer* (+)-**7** *and MTPA Esters* **4**, **5**, **8**, *and* **9**[a]). Arbitrary numbering.

	$\Delta\delta$(**2** – **1**)	**1**	**2**	**7**	$\Delta\delta$(**2** – **7**)	**4**	**5**	$\Delta\delta$(**4** – **5**)	**8**	**9**	$\Delta\delta$(**8** – **9**)
H_α–C(2)	–	2.30	2.31	2.39	*–0.08*	2.27	2.28	–	2.37	2.35	*+0.02*
H_β–C(2)	–	2.52	2.52	2.56	*–0.04*	2.46	2.49	*–0.03*	2.56	2.55	–
H–C(3)	*+0.17*	4.00	4.17	3.71	*+0.46*	3.88	3.97	*–0.09*	3.81	3.81	–
H_α–C(4)	–	1.52	1.52	1.21	*+0.31*	1.56	1.59	*–0.03*	1.44	1.36	*+0.08*
H_β–C(4)	*–0.13*	1.85	1.72	2.03	*–0.31*	1.92	1.85	*+0.07*	2.14	2.07	*+0.07*
H–C(5)	*–0.93*	5.24	4.31	3.88	*+0.43*	5.46	5.48	*–0.02*	5.20	5.21	–
H_α–C(6)	–	1.59	*ca.* 1.60	1.29	*+0.31*	1.67	1.62	*+0.05*	1.42	1.50	*–0.08*
H_β–C(6)	–	1.71	*ca.* 1.60	1.92	*–0.32*	1.76	1.79	*–0.03*	1.95	2.06	*–0.11*
H–C(7)	*+0.16*	3.55	*ca.* 3.71	3.21	*+0.50*	3.54	3.44	*+0.10*	3.31	3.32	–
H_α–C(8)	–	1.93	1.94	2.03	*–0.09*	1.96	1.92	*+0.04*	1.99	2.01	*–0.02*
H_β–C(8)	–	1.19	1.18	1.23	*–0.05*	1.14	1.09	*+0.05*	1.23	1.25	*–0.02*
H–C(9)	*+0.02*	3.52	3.54	3.51	*+0.03*	3.46	3.44	*+0.02*	3.48	3.49	–
MeO–C(9)	–	3.34	3.35	3.35	–	3.33	3.32	–	3.32	3.33	–
H_β–C(10)	*+0.07*	2.37	2.44	2.36	*+0.08*	2.26	2.20	*+0.06*	2.34	2.34	–
H_αC(10)	–	0.98	0.99	1.00	–	0.97	0.94	*+0.03*	1.00	1.00	–
H–C(11)	–	3.88	3.89	3.88	–	3.84	3.84	–	3.87	3.87	–
H–C(12)	–	*ca.* 1.50	*ca.* 1.52	1.52	–	*ca.* 1.46	*ca.* 1.47	–	*ca.* 1.51	*ca.* 1.51	–
Me–C(12)	–	1.15	1.16	1.16	–	1.14	1.14	–	1.15	1.15	–
H–C(15)	*+0.04*	3.55	3.59	3.51	*+0.08*	3.42	3.44	*–0.02*	*ca.* 3.50	3.49	–
H_α–C(16)	–	1.64	*ca.* 1.63	1.64	–	1.63	1.58	*+0.05*	1.65	1.65	–
H_β–C(16)	–	*ca.* 1.73	*ca.* 1.72	1.71	–	1.74	1.68	*+0.06*	1.74	1.73	–
H–C(17)	–	5.34	*ca.* 5.35	5.35	–	5.27	5.27	–	5.33	*ca.* 5.32	–
H–C(18)	–	5.34	*ca.* 5.36	5.35	–	5.35	5.33	*+0.02*	5.33	5.33	–
H–C(19)	–	5.69	5.70	5.70	–	5.70	5.68	*+0.02*	5.70	5.70	–
2 H–C(20)	–	1.83	1.84	1.84	–	1.84	1.82	*+0.02*	1.83	1.83	–
	+0.02	1.89	1.91	1.90	–	1.91	1.89	*+0.02*	1.90	1.90	–
H–C(21)	–	1.58	1.58	1.59	–	1.59	1.59	–	1.58	1.58	–
3 H–C(22)	*+0.02*	0.83	0.85	0.85	–	0.84	0.83	–	0.84	0.84	–
3 H–C(23)											

[a]) A dash (–) indicates $+0.01 \geq \Delta\delta \geq -0.01$.

((+)-**1**), and provided also useful information about the relative configurations. Thus, small but significant variations in chemical shifts of H_β–C(10) and H–C(15) among (+)-**1**, (+)-**2**, and (+)-**7** on the one side, as well as between the MTPA esters **4** and **5** with O_{ax}–C(5) on the other side (but not between the equatorial analogues **8** and **9**) (*Table 2*), suggested that H_β–C(10), H–C(15), and O_{ax}–C(5) lie on the same side of the mean plane of the macrocycle. Based on the (5*R*)-configuration, this establishes the absolute configuration at all chiral centres of leucascandrolide A as shown in (+)-**1**.

2.4. *Conformation.* Molecular-mechanics calculations for the macrolide portion (+)-**2** of leucascandrolide A were carried out allowing for rotation of dihedral angles O–C(9)–C(10)–C(11) from 170 to 10°, and O=C(1)–O–C(17) from 40 to –40°. In the minimum-strain conformation represented in the *Figure*, the above dihedral angles take the values 58.7 and –3.3°, respectively, in good agreement with the observed NMR coupling values.

2.5. *Bioactivity.* The sponge raw extracts proved strongly antimicrobial, toxic, and cytotoxic (see *Exper. Part*). These activities were traced to mainly leucascandrolide A

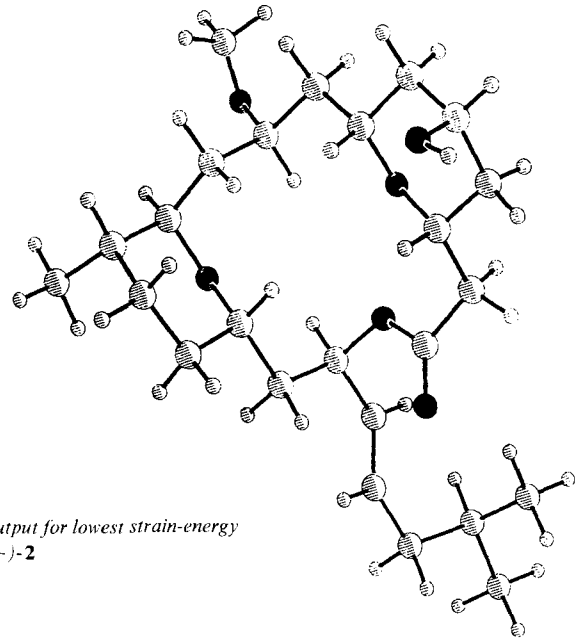

Figure. *MM Output for lowest strain-energy conformer of (+)-***2**

((+)-**1**), which showed strong cytotoxic activity *in vitro* on KB cells and less marked action on P388 cells, as well as very strong inhibition of *Candida albicans*. The latter is interesting in view of growing concern by AIDS-infected people about this animal-pathogenic yeast.

Having found a clean method of separation of the macrolide part (+)-**2** from the oxazole-bearing chain part **3**, it was interesting to compare their bioactivities. The data in the *Exper. Part* show that the macrolide moiety is essential for cytotoxic activity, while the oxazole-containing side chain, *per se* more fungistatic than fungicide, seriously contributes to the antifungal properties of leucascandrolide A.

3. Conclusions. – In the complex ensemble of marine macrolides, leucascandrolide A ((+)-**1**) compares best to both polyoxygenated macrolides of the sphinxolide type [8] and polycycloether macrolides of the halichondrin type [9]. Even with respect to these, however, leucascandrolide A is distinguished for a single C_1 branching *vs.* extensive 1,3-dioxygenation in the macrolide part and an unusual oxazole-bearing side chain of uncertain biogenesis. Finding such a structurally unusual and highly biologically active macrolide in a calcareous sponge, a class of sponges so far only known to give scarcely bioactive 2-aminoimidazoles, raises questions about the biogenesis of this metabolite and the ecology of the sponge. *L. caveolata* belongs to the same subclass, Calcinea, and in one case even to the same order, Leucettida, as calcareous sponges that contain 2-aminoimidazoles. On these bases, one might reasonably suspect that leucascandrolide A ((+)-**1**) originates from special microbes present in *L. caveolata*. Though well possible, evidence is lacking at present; what we know is that the *L. caveolata* main symbiont, the cyanobacterium *Aphanocapsa feldmanni*, occurs widely in calcareous sponges, none of which, except *L. caveolata*, are known to produce macrolides.

Whatever its origin, leucascandrolide A, as a powerful antifungal agent, might have adaptive value, playing a defensive role in *L. caveolata*. However, why just this calcareous sponge needs such a defence, while other taxonomically close sponges from the same tropical waters do not, transcends any possible rationalization at this stage of knowledge. With the aim to answer such questions and, hopefully, to enlarge our knowledge, and to exploit such powerfully biologically active metabolites as encountered here, we are embarked in a program of searching *L. caveolata* in other areas of the New Caledonian coral reef. In any event, present findings are likely to revitalize interest in calcareous sponges and their symbionts.

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Experimental Part

1. *General.* All evaporations were carried out under reduced pressure below 40°. Yields are given in the captions to schemes. Flash chromatography (FC): *Merck RP-18 LiChroprep* (40–65 μm). TLC: *Merck* silica gel *60 PF_{254}* plates. HPLC: *Reinin Dynamax 60A C-18* (8 μm); 25 × 1-cm column, solvent flux 3 ml/min; UV monitoring at λ 254 nm. UV: *Perkin-Elmer-Lambda-3* spectrophotometer, λ_{max} in nm, ε in $mol^{-1}\,l\,cm^{-1}$. Polarimetric data: *JASCO-DIP-181* polarimeter; $[\alpha]_D$ in dm^{-1} deg ml g^{-1}. IR: *Philips-Pye-Unicam-SP3-200S* spectrophotometer; $\tilde{\nu}_{max}$ in cm^{-1}. NMR: *Varian-XL-300* (^1H at 299.94 MHz, ^{13}C at 75.4 MHz) and *Varian-VXR-600* (^1H at 600 MHz) spectrometer; assignments confirmed by one-bond $^1H,^{13}C$ correlation data (HMQC) [10]; δ in ppm rel. to internal $SiMe_4$ (= 0 ppm) and J in Hz; long-range $^1H,^{13}C$ correlation data (HMBC) [11]; ROESY, [12]; DEPT, [13]; DQ-COSY, [14]; 'small' indicates $J < 0.5$ Hz. MS (*m/z* (%)): electron ionization; *Kratos-MS80* spectrometer with home-built data system. Molecular-mechanics calculations: PCMOD 4.0 for *Windows*™, based on MMX force field, as described in program instructions by *Serena Software*, Bloomington, Indiana, 1993.

2. *Collection and Isolation.* The sponge (R1485, 689M) was collected in September 1989 (3 kg of fresh weight = 200 g of freeze-dried) and July 1992 (40 g of freeze-dried) by scuba diving along the external slope of the Passe de Nakety, eastern coast of New Caledonia, at depths 20–40 m. The sponge was immediately deep frozen and then freeze-dried. The sponge, an arborescent brownish mass of tubules when alive, 5 × 5 to 25 × 25 cm overall dimensions, of distribution limited to New Caledonia, remained long a taxonomic puzzle, until it was defined as a new genus, *Leucascandra caveolata* BOROJEVIC and KLAUTAU, family Leucascidae Dendy 1892, order Leucettida, subclass Calcinea, class Calcarea. The sponge mass turned to white when immersed in EtOH, which became green due to the symbiont *Aphanocapsa feldmanni*, a cyanobacterium of worldwide diffusion in calcareous sponges. We regularly encountered the sponge at the above described location, until in April 1995, only a few, quite small specimens could be found. Since calcareous sponges are opportunistic and their life cycle is limited to only 2–3 years, disappearance of *L. caveolata* from our fishing spots is likely to result from a major ecological change. Since the Passe of Nakety, like all passages to the New Caledonian lagoons, is in front of water effluents, scarcity of rain during the last three years might be at the basis of an ecological change. A 240-g portion of freeze-dried sponge was CH_2Cl_2 extracted (3 × 700 ml). Evaporation of the solvent gave 1.8 g of residue that was subjected to FC (packing with H_2O/MeOH 1:1, elution with MeOH). Central fractions were evaporated to give 0.8 g of residue that was subjected to HPLC (MeOH/H_2O 9:1), collecting (+)-**1** (70 mg) at t_R 10.5 min.

(1R,3R,5R,7R,9R,13R,15S,18S)-3-Methoxy-18-methyl-13-[(E)-4-methylpent-1-enyl]-11-oxo-12,19,20-trioxatricyclo[13.3.1.1^{5,9}]icos-7-yl (Z)-5-{2-{(Z)-3-[(Methoxycarbonyl)amino]prop-1-enyl}oxazol-4-yl}pent-2-enoate ((+)-**1**): Colorless solid. $[\alpha]_D^{20} = +41$, $[\alpha]_{577} = +48$, $[\alpha]_{546} = +54$, $[\alpha]_{435} = +88$, $[\alpha]_{365} = +136$. UV (EtOH): 205 (15000), 262 (11000). IR (liq. film): 2980*s*, 1740*s*, 1540*w*, 1190*m*. ^{1}H-NMR (C_5D_5N)[2]: 2.46 (*dd*, $J_{gem} = 13.2$, $J(2\alpha,3) = 11.5$, H_α–C(2)); 2.71 (*dd*, $J_{gem} = 13.2$, $J(2\beta,3) = 3.6$, H_β–C(2)); 4.32 (*dddd*, $J(3,2\alpha) = 11.5$, $J(3,2\beta) = 3.6$, $J(3,4\alpha) = 11.5$, $J(3,4\beta) = 2$, H–C(3)); 1.51 (*ddd*, $J_{gem} = 14$, $J(4\alpha,3) = 11.5$, $J(4\alpha,5) = 3$, H_α–C(4)); 1.96 (br. *d*, $J_{gem} = 14$, $J(4\beta,3) = 2$, $J(4\beta,5) = 2.9$, $J(4\beta,6\beta) = 2.8$, H_β–C(4)); 5.41 (*m*, $J(5,6\alpha) = 3$, $J(5,6\beta) = 2.9$,

$J(5,4\alpha) = 3$, $J(5,4\beta) = 2.9$, H–C(5)); 1.62 (*ddd*, $J_{gem} = 14$, $J(6\alpha,7) = 11.5$, $J(6\alpha,5) = 3$, H_α–C(6)); 1.89 (br. *d*, $J_{gem} = 14$, $J(6\beta,7) = 2$, $J(6\beta,5) = 2.9$, $J(6\beta,4\beta) = 2.8$, H_β–C(6)); 3.87 (br. *dd*, $J(7,6\alpha) = 11.5$, $J(7,6\beta) = 2$, $J(7,8\alpha) = 11$, $J(7,8\beta) = 3$, H–C(7)); 2.06 (*ddd*, $J_{gem} = 12.0$, $J(8\alpha,7) = 11$, $J(8\alpha,9) = 1.8$, H_α–C(8)); 1.30 (*ddd*, $J_{gem} = 12$, $J(8\beta,9) = 10.8$, $J(8\beta,7) = 3$, H_β–C(8)); 3.90 (br. *dd*, $J(9,8\alpha) = 1.8$, $J(9,8\beta) = 10.8$, $J(9,10\alpha) = 11.4$, $J(9,10\beta)$ small, H–C(9)); 3.39 (*s*, MeO–C(9)); 1.08 (*ddd*, $J_{gem} = 14.4$, $J(10\alpha,9) = 11.4$, $J(10\alpha,11) = 2.4$, H_α–C(10)); 2.50 (br. *dd*, $J_{gem} = 14.4$, $J(10\beta,11) = 11.4$, $J(10\beta,9)$ small, H_β–C(10)); 4.09 (br. *d*, $J(11,10\alpha) = 2.4$, $J(11,10\beta) = 11.4$, $J(11,12) = 2$, H–C(11)); 1.42 br. *m*, $J(12,24) = 7.2$, $J(12,11) = 2$, $J(12,13\alpha) = 2$, $J(12,13\beta) = 4$, H–C(12)); 1.13 (*d*, $J(24,12) = 7.2$, 3 H–C(24)); 1.30 (br. *d*, $J_{gem} = 14$, $J(13\alpha,12) = 2$, $J(13\alpha,14\alpha) = 4$, $J(13\alpha,14\beta) = 2$, H_α–C(13)); 1.85 (*dddd*, $J_{gem} = 14$, $J(13\beta,12) = 4$, $J(13\beta,14\alpha) = 13$, $J(13\beta,14\beta) = 4.5$, H_β–C(13)); 1.47 (*dddd*, $J_{gem} = 14$, $J(14\alpha,13\beta) = 13$, $J(14\alpha,13\alpha) = 4$, $J(14\alpha,15) = 10.8$, H_α–C(14)); 1.24 (br. *d*, $J_{gem} = 14$, $J(14\beta,13\beta) = 4.5$, $J(14\beta,13\alpha) = 2$, $J(14\beta,15) = 3$, H_β–C(14)); 3.81 (br. *dd*, $J(15,14\alpha) = 10.8$, $J(15,14\beta) = 3$, $J(15,16\alpha) = 10.2$, $J(15,16\beta) = 1.5$, H–C(15)); 1.78 (*ddd*, $J_{gem} = 14.4$, $J(16\alpha,15) = 10.2$, $J(16\alpha,17) = 1.8$, H_α–C(16)); 1.89 (submerged, $J_{gem} = 14.4$, $J(16\beta,17) = 11.7$, $J(16\beta,15) = 1.5$, H_β–C(16)); 5.76 (br. *dd*, $J(17,16\alpha) = 1.8$, $J(17,16\beta) = 11.7$, $J(17,18) = 6.9$, $J(17,19)$ small, H–C(17)); 5.59 (*ddt*, $J(18,19) = 15.0$, $J(18,17) = 6.9$, $J(18,20) = 1.2$, H–C(18)); 5.82 (br. *dt*, $J(19,18) = 15.0$, $J(19,20) = 7.2$, $J(19,17)$ small, H–C(19)); 1.88 (br. *dd*, $J(20,19) = 7.2$, $J(20,18) = 1.2$, $J(20,21) = 6.6$, 2 H–C(20)); 1.55 (*m*, $J(21,20) = J(21,22) = J(21,23) = 6.6$, H–C(21)); 0.82 (*d*, $J(22,21) = 6.6$, 3 H–C(22)); 0.83 (*d*, $J(23,21) = 6.6$, 3 H–C(23)); 5.97 (*dt*, $J(2',3') = 11.0$, $J(2',4') = 1.7$, H–C(2')); 6.31 (*dt*, $J(3',2') = 11.0$, $J(3',4') = 7.4$, H–C(3')); 3.20 (*ddt*, $J(4',2') = 1.7$, $J(4',3') = 7.4$, $J(4',5') = 7.2$, 2 H–C(4')); 2.75 (br. *t*, $J(5',4') = 7.2$, $J(5',7')$ small, 2 H–C(5')); 7.64 (br. *s*, $J(7',5')$ small, H–C(7')); 6.40 (*dt*, $J(9',10') = 12.0$, $J(9',11') = 2.0$, H–C(9')); 6.27 (*dt*, $J(10',9') = 12.0$, $J(10',11') = 6.0$, H–C(10')); 4.79 (*ddd*, $J(11',10') = 6.0$, $J(11',9') = 2.0$, $J(11',NH–C(11')) = 5.5$, 2 H–C(11')); 8.35 (br. *t*, $J(NH–C(11'),11') = 5.5$, NH–C(11')); 3.74 (*s*, MeOOCN). ROESY[2]): H_α–C(2)/H_α–C(4); H_β–C(2)/H–C(3); H–C(3)/H–C(7); H–C(3)/H_β–C(4); H_β–C(6)/H–C(7); H_α–C(6)/H_α–C(8); H–C(7)/H_β–C(10); H–C(7)/H_β–C(8); H_α–C(8)/MeO–C(9); H_α–C(8)/H–C(9); H–C(9)/H–C(17); H_β–C(10)/H–C(15); H_α–C(10)/H–C(11); H_β–C(10)/H_β–C(13); H_α–C(10)/H–C(12); H–C(11)/H–C(12); H–C(11)/3 H–C(24); H–C(11)/H_β–C(13); 3 H–C(24)/H_α–C(14); H_β–C(13)/H–C(15); H_β–C(14)/H–C(15); H_α–C(14)/H–C(16); H_α–C(16)/H–C(17). MS: 700 (3.4, $M^{+\cdot}$), 685 (0.8, $[M - Me]^+$), 612 (1), 590 (7, $[M - C_8H_{14}]^{+\cdot}$), 558 (2, $[590 - MeOH]^+$), 502 (1), 420 (5, $[M - C_{13}H_{16}N_2O_5]^{+\cdot}$), 311 (6), 279 (9), 44 (100). HR-MS: 700.39160 ± 0.01 ($[C_{38}H_{56}N_2O_{10}]^{+\cdot}$, calc. 700.39349), 590.28322 ± 0.01 ($[C_{30}H_{42}N_2O_{10}]^+$, calc. 590.28394), 420.28749 ± 0.005 ($[C_{25}H_{40}O_5]^{+\cdot}$, calc. 420.28757).

3. *Transesterification of (+)-***1**. A mixture of (+)-**1** (9.8 mg, 0.014 mmol) and Na_2CO_3 in MeOH (2 ml) was stirred for 2 days at r.t. and then evaporated. The residue was subjected to prep. TLC (petroleum ether/AcOEt 4:6): (+)-**2** (4 mg, R_f 0.29), **3** (2 mg, R_f 0.63), and unreacted (+)-**1** (2 mg, R_f 0.52).

*(1*R*,3*R*,5*R*,7*R*,9*R*,13*R*,15*S*,18*S*)-7-Hydroxy-3-methoxy-18-methyl-13-[(*E*)-4-methylpent-1-enyl]-12,19,20-trioxatricyclo[13.3.1.1^{5,9}]icosan-11-one* ((+)-**2**): $[\alpha]_D^{20} = +24$ (EtOH, $c = 0.05$). ^{1}H-NMR (C_5D_5N; *J*'s identical to those of (+)-**1**[2]): 2.52 (*dd*, H_α–C(2)); 2.72 (*dd*, H_β–C(2)); 4.68 (*dddd*, H–C(3)); 1.53 (*ddd*, H_α–C(4)); 1.96 (br. *d*, H_β–C(4)); 4.46 (*m*, H–C(5)); 1.66 (*ddd*, H_α–C(6)); 1.93 (br. *d*, H_β–C(6)); 4.22 (br. *dd*, H–C(7)); 2.15 (*ddd*, H_α–C(8)); 1.34 (*ddd*, H_β–C(8)); 3.96 (br. *dd*, H–C(9)); 3.41 (*s*, MeO–C(9)); 1.07 (*ddd*, H_α–C(10)); 2.53 (br. *dd*, H_β–C(10)); 4.10 (br. *d*, H–C(11)); 1.37 (submerged, H–C(12)); 1.11 (*d*, 3 H–C(24)); 1.22 (br. *d*, H_α–C(13)); 1.68 (*dddd*, H_β–C(13)); 1.38 (*dddd*, H_α–C(14)); 1.16 (br. *d*, H_β–C(14)); 3.78 (br. *dd*, H–C(15)); 1.76 (*ddd*, H_α–C(16)); 1.88 (*ddd*, H_β–C(16)); 5.77 (br. *dd*, H–C(17)); 5.58 (*ddt*, H–C(18)); 5.81 (*ddt*, H–C(19)); 1.87 (br. *dd*, 2 H–C(20)); 1.54 (*m*, H–C(21)); 0.81 (*d*, 3 H–C(22)); 0.82 (*d*, 3 H–C(23)). MS: 438 (9, $M^{+\cdot}$), 420 (3, $[M - H_2O]^{+\cdot}$), 406 (8, $[M - MeOH]^+$), 328 (26), 296 (12), 279 (14), 81 (100). HR-MS: 438.29791 ± 0.005 ($[C_{25}H_{42}O_6]^{+\cdot}$, calc. 438.29813), 328.18857 ± 0.005 ($[C_{17}H_{28}O_6]^{+\cdot}$, calc. 328.18858).

Methyl (Z)-5-{2-[(Z)-3-[(Methoxycarbonyl)amino]prop-1-enyl]oxazol-4-yl}pent-2-enoate (**3**): ^{1}H-NMR (C_5D_5N)[2]): 3.63 (*s*, MeO–C(1')); 5.91 (*dt*, H–C(2')); 6.27 (*dt*, H–C(3')); 3.14 (*ddt*, 2 H–C(4')); 2.68 (br. *t*, 2 H–C(5')); 7.60 (br. *s*, H–C(7')); 6.38 (*dt*, H–C(9')); 6.25 (*dt*, H–C(10')); 4.79 (*ddd*, 2 H–C(11')); 8.46 (br. *t*, NH–C(11')); 3.74 (*s*, MeOOCN). MS: 294 (100, $M^{+\cdot}$), 262 (57, $[M - MeOH]^{+\cdot}$), 233 (74), 195 (41), 149 (38), 67 (55). HR-MS: 294.12154 ± 0.005 ($[C_{14}H_{18}N_2O_5]^{+\cdot}$, calc. 294.12157).

4. *MTPA Esters* **4** *and* **5**. A soln. of (+)-**2** (2 mg, 4.6 µmol) and (+)-(*S*)-MTPA-Cl (3.5 mg, 2.6 µl, 13.8 µmol) in dry pyridine (40 µl) was allowed to stand at r.t. for 5 h and then evaporated. The residue was subjected to TLC (petroleum ether/AcOEt/i-PrNH$_2$ 4:6:0.1): **5** (0.5 mg, R_f 0.78) and **2** (1.4 mg, R_f 0.38).

Using (−)-(*R*)-MTPA-Cl, 0.4 mg of **4** were analogously obtained.

5. *Oxidation of (+)-***2**: *(1*R*,3*R*,5*R*,9*R*,13*R*,15*S*,18*S*)-3-Methoxy-18-methyl-13-[(*E*)-4-methylpent-1-enyl]-12,19,20-trioxatricyclo[13.3.1.1^{5,9}]icosane-7,11-dione* ((+)-**6**). A mixture of (+)-**2** (6.6 mg, 15 μmol) and pyridinium chlorochromate (9.7 mg, 45 μmol) in 1 ml of CH_2Cl_2 was stirred at r.t. for 2 h and then evaporated. The residue was subjected to TLC (petroleum ether): (+)-**6** (4.6 mg). R_f 0.70. $[\alpha]_D^{20} = +50$ (EtOH, $c = 0.38$). ^{1}H-NMR (C_5D_5N; *J*'s identical to those of [+]-**1**, unless otherwise specified)[2]): 2.59 (*dd*, H_α–C(2)); 2.77 (*dd*, H_β–C(2)); 4.13 (*dddd*, H–C(3)); 2.33 (*dd*, $J_{gem} = 14.5$, $J(4\alpha,3) = 11.5$, H_α–C(4)); 2.53 (*ddd*, $J_{gem} = 14.5$, $J(4\beta,3) = 2.7$, $J(4\beta,6\beta) = 1.3$, H_β–C(4)); 2.45 (superimposed, 2 H–C(6)); 3.69 (br. *dd*, H–C(7)); 2.17 (*ddd*, H_α–C(8)); 1.34 (*ddd*, H_β–C(8)); 3.87 (br. *dd*, H–C(9)); 3.41 (*s*, MeO–C(9)); 1.13 (*ddd*, H_α–C(10)); 2.46 (br. *dd*, H_β–C(10)); 4.11 (br. *d*, H–C(11)); 1.45 (br. *m*, H–C(12)); 1.14 (*d*, 3 H–C(24)); 1.37 (submerged, H_α–C(13)); 1.91 (submerged, H_β–C(13)); 1.47 (*dddd*, H_α–C(14)); 1.35 (submerged, H_β–C(14)); 3.76 (br. *dd*, H–C(15)); 1.80 (*ddd*, H_α–C(16)); 1.94 (*ddd*, H_β–C(16)); 5.76 (br. *ddd*, H–C(17)); 5.58 (*ddt*, H–C(18)); 5.83 (br. *dt*, H–C(19)); 1.88 (br. *dd*, 2 H–C(20)); 1.55 (*m*, H–C(21)); 0.81 (*d*, 3 H–C(22)); 0.82 (*d*, 3 H–C(23)). MS: 436 (10, $M^{+\cdot}$), 404 (6, $[M - MeOH]^{+\cdot}$), 326 (14), 294 (12), 223 (17), 157 (42), 95 (100). HR-MS: 436.28204 ± 0.005 ($[C_{25}H_{40}O_6]^{+\cdot}$, calc. 436.28248), 404.25608 ± 0.005 ($[C_{24}H_{36}O_5]^{+\cdot}$, calc. 404.25627).

6. *Hydride Reduction of (+)-***6**: *(1*R*,3*R*,5*R*,7*S*,9*R*,13*R*,15*S*,18*S*)-7-Hydroxy-3-methoxy-18-methyl-13-[(*E*)-4-methylpent-1-enyl]-12,19,20-trioxatricyclo[13.3.1.1^{5,9}]icosan-11-one* ((+)-**7**). A mixture of (+)-**6** (4.6 mg, 10 μmol) and excess $NaBH_4$ in 0.5 ml of abs. EtOH was stirred at r.t. for 1 h. Excess $NaBH_4$ was then quenched with AcOH and the mixture subjected to TLC (petroleum ether/AcOEt 2:3): (+)-7/(+)-**2** 95:5 (4 mg, R_f 0.26). (+)-**7**: $[\alpha]_D^{20} = +58$ (EtOH, $c = 0.1$). ^{1}H-NMR (C_5D_5N; *J*'s identical to those of (+)-**1**, unless otherwise specified)[2]): 2.55 (*dd*, H_α–C(2)); 2.72 (*dd*, H_β–C(2)); 3.89 (br. *dd*, H–C(3)); 1.49 (*ddd*, $J_{gem} = 13$, $J(4\alpha,3) = 11.5$, $J(4\alpha,5) = 11$, H_α–C(4)); 2.23 (br. *d*, $J_{gem} = 13$, $J(4\beta,3) = 2$, $J(4\beta,5) = 5$, H_β–C(4)); 4.11 (*m*, $J(5,6\alpha) = 11$, $J(5,6\beta) = 5$, $J(5,4\alpha) = 11$, $J(5,4\beta) = 5$, $J(5,OH) = 5$, H–C(5)); 6.60 (*d*, $J(OH,5) = 5$, OH–C(5)); 1.61 (*ddd*, $J_{gem} = 12$, $J(6\alpha,7) = 11.5$, $J(6\alpha,5) = 11$, H_α–C(6)); 2.17 (br. *d*, $J_{gem} = 12$, $J(6\beta,7) = 2$, $J(6\beta,5) = 5$, H_β–C(6)); 3.48 (br. *dd*, H–C(7)); 2.20 (br. *dd*, H_α–C(8)); 1.35 (br. *dd*, H_β–C(8)); 3.92 (br. *dd*, H–C(9)); 3.38 (*s*, MeO–C(9)); 1.12 (*ddd*, H_α–C(10)); 2.56 (br. *dd*, H_β–C(10)); 4.12 (br. *d*, H–C(11)); 1.45 (submerged, H–C(12)); 1.15 (*d*, 3 H–C(24)); 1.33 (submerged, H_α–C(13)); 1.88 (submerged, H_β–C(13)); 1.49 (*dddd*, H_α–C(14)); 1.35 (submerged, H_β–C(14)); 3.82 (br. *dd*, H–C(15)); 1.81 (*ddd*, H_α–C(16)); 1.95 (*ddd*, H_β–C(16)); 5.77 (br. *dd*, H–C(17)); 5.60 (*ddt*, H–C(18)); 5.82 (br. *dt*, H–C(19)); 1.88 (br. *dd*, 2 H–C(20)); 1.54 (*m*, H–C(21)); 0.81 (*d*, 3 H–C(22)); 0.82 (*d*, 3 H–C(23)). MS: 438 (7, $M^{+\cdot}$), 406 (7, $[M - MeOH]^{+\cdot}$), 328 (23), 296 (9), 279 (10), 81 (100).

7. *MTPA Esters* **8** *and* **9**. A soln. of (+)-7/(+)-**2** (see *Exper. 6*; 1.5 mg, 3.4 μmol) and (–)-(*R*)-MTPA-Cl (1.92 μl, 10.2 μmol) in dry pyridine (40 μl) was allowed to stand at r.t. overnight and then evaporated. The residue was subjected to TLC (petroleum ether/AcOEt 4:1): **8** (1.2 mg, R_f 0.62).

Using (+)-(*S*)-MTPA-Cl, 1.2 mg of **9** were analogously obtained.

8. *Biological Assays*. Both the lipophilic and aq. extracts from freeze-dried *L. caveolata* proved strongly inhibitory of phytopatogenic fungi *Fusarium oxysporum*, *Helminthosporium sativum*, *Phytophtora hevea*, *Botrytis cinerea*, and *Pyricularia oryzae*, as well as of animal-pathogenic yeast *Candida albicans*. The lipophilic extract proved also strongly cytotoxic to both KB throat epithelial cancer cell lines and P388 murine leukemia cell lines, while aq. extracts were only KB active. The lipophilic extracts proved also strongly toxic to the crustacean *Artemia salina*. Disks of 6-mm diameter were used for antifungal/antiyeast assays.

Pure compounds gave the following results. Cytotoxicity assays of (+)-**1** at *Rhône-Poulenc* gave IC_{50} 0.05 and 0.25 (μg/ml) with KB and P388 cells, respectively. Comparative assays, run simultaneously and in duplicate, at ORSTOM, Nouméa, with KB cell lines on the same plate, gave for (+)-**1** 100% toxicity at 10–0.1 μg/ml, for either (+)-**2** or **3** 100% toxicity at 10–0.5 μg/ml and negligible toxicity at 0.1 μg/ml. Similarly, in experiments at ORSTOM with *C. albicans*, the inhibition diameter [mm]/μg per disk was for (+)-**1** 26/40, 23/20, and 20/10, for (+)-**2** 20/40, 18/20, and 15/10, and for **3** 17/40, 13/20, and 10/10; in the latter case, the action proved to be more fungistatic than fungitoxic.

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