Separatum
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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)

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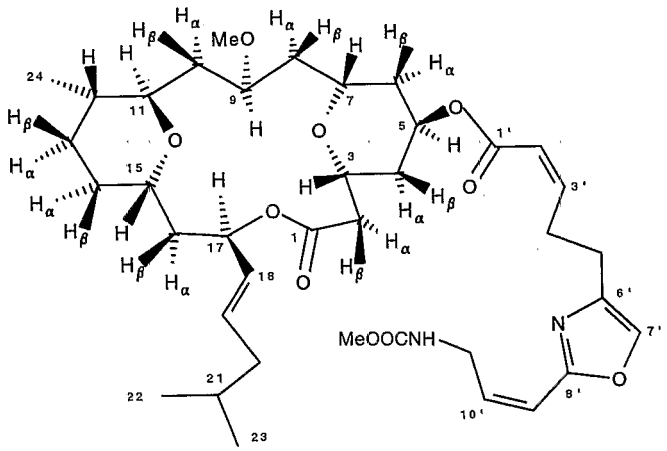
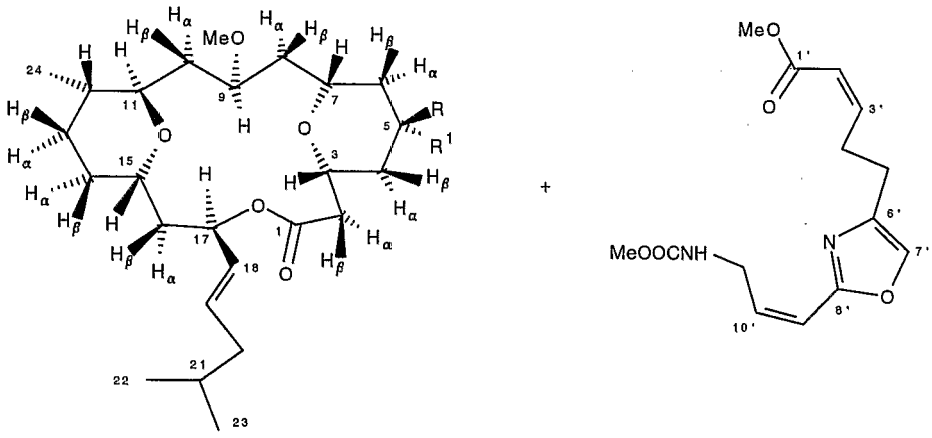
Scheme



a)



(+)-**2** R = OH, R^1 = H
4 R = O[(*S*)-MTPA], R^1 = H
5 R = O[(*R*)-MTPA], R^1 = H
(+)-**6** R, R^1 = O
(+)-**7** R = H, R^1 = OH
8 R = H, R^1 = O-(*S*)-MTPA
9 R = H, R^1 = O-(*R*)-MTPA

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

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*).

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)

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	

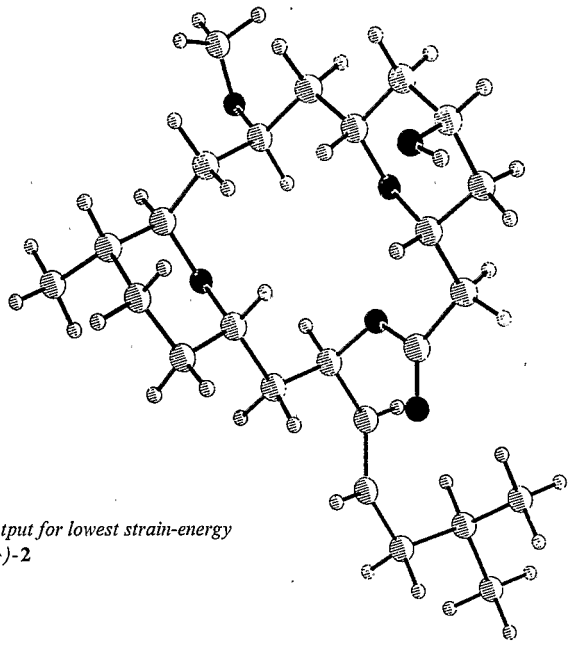


Figure. *MM Output for lowest strain-energy conformer of (+)-2*

((+)-**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. aspeculata* belongs to the same subclass, Calcinea, and in one

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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$J(5,4\alpha) = 3$, $J(5,4\beta) = 2.9$, 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 ($[C_{30}H_{42}N_2O_{10}]^{+\cdot}$, 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, $[M - MeOH]^{+\cdot}$), 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

5. Oxidation of (+)-2: (1R,3R,5R,9R,13R,15S,18S)-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: (1R,3R,5R,7S,9R,13R,15S,18S)-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

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