A Novel Type of a Second Epoxy Bridge in Eunicellane Diterpenes: Isolation and Characterization of Massileunicellins A – C from the Gorgonian *Eunicella cavolinii*

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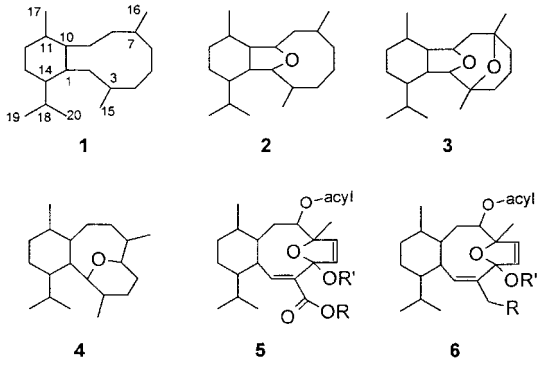
Massileunicellins A (**7**), B (**9**), and C (**11**) – which show a novel type of a second epoxy bridge in eunicellane diterpenes – were isolated from the gorgonian *Eunicella cavoliniii* collected near Marseille. Structural assignments are based on NMR and MS data of these compounds and of their ketal derivatives **8**, **10**, and **12**. Negligible activity of the massileunicellins on L1210 and KB tumor cell lines, and similar results for related known compounds, cast doubt on high cytotoxicity reported for the latter by other authors.

1. Introduction. – Numbers of diterpenoids isolated from gorgonians and alcyonarians may be imagined to derive from 2,11-cyclization of cembrane biogenetic precursors. Apart from a few non-epoxy-bridged compounds (see **1**) [1], an epoxy bridge was commonly observed between C(2) and C(9) (see **2**). The first such compound, eunicellin, was isolated from the gorgonian *Eunicella singularis* (= *Eunicella stricta*) from East-Pyrenean waters [2]. Variants on the eunicellin structural theme are cladiellins [3a] and related diterpenes [3b – g], litophynins [4a] and litophynols [4b], ophirin [5a] and astrogorgin [5b], palmonins [6], labiatins B and C [7], and labiatamides [7], as well as sclerophytins C-F[8].



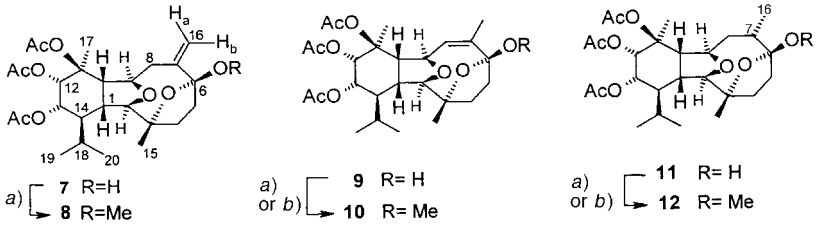
1 **2** **3** **4** **5** **6**

Fig. 1. *Known types of epoxy bridges in 2,11-cyclized cembranoids* (see **1**), *isolated from gorgonians and alcyonaceans: eunicellins and cladiellins* (**2**), *sclerophytins* (**3**), *labiatins* (**4**), *sarcodictyins* (**5**), *and eleutherobins, valdivones, and eleuthosides* (**6**). Arbitrary numbering.

Sclerophytins A-B (**3**) bear an additional 3,7-epoxy bridge [9], whereas labiatin A (**4**) deviates from this pattern by having a single 2,6-epoxy bridge [7]. Finally, a hemiacetal or hemiketal 4,7-bridge characterizes sarcodictyins (**5**, R′ = OH, R = Me, Et) [10], eleutherobin (**6**, R′ = Me, R = *O*-glycoside) [11], eleuthosides (**6**, R′ = H, R = *O*-glycoside) [12], and valdivones (**6**, R′ = R = H) [13] (*Fig. 1*)[1].

We report here on a new epoxy-bridging type for eunicellane diterpenes, massileunicellins A (**7**), B (**9**), and C (**11**) (*Scheme*), isolated from *Eunicella cavolinii* collected near Marseille and named for its ancient name, Massilia. A collection of this gorgonian from the Bay of Naples seems to have been studied previously with different results [15].

Scheme. *A New Type of an Epoxy Bridge in 2,11-Cyclized Cembranoids Isolated from the Gorgonian* Eunicella cavolinii: *massileunicellins A* (**7**), *B* (**9**), *and C* (**11**). Arbitrary numbering as in *Fig 1*; for systematic names, see *Exper. Part. a*) $CDCl_3$/MeOH, conc. to dryness, 98%; *b*) PPTS (pyridinium *p*-toluenesulfonate, cat.), MeOH, r.t., 15 min; 94%.



2. Results and Discussion. – The composition $C_{26}H_{38}O_9$ for massileunicellin A (**7**) is based on the observation of the protonated molecular ion in the FAB-MS as well as on HR-EI-MS measurements on fragment ions *m/z* 476 and 434, which derive by loss of a molecule of H_2O or AcOH, respectively, from the molecular ion. This agrees with the composition $C_{27}H_{40}O_9$ derived from both HR-EI-MS measurements on the molecular ion of methyl derivative **8** and 1D/2D-NMR spectra of **7** (*Table*), which fit for an eunicellan-type diterpene.

The substitution pattern on the six-membered ring of **7** is suggested by the following NMR observations (*Table*): *i*) three *s* at δ(H) 2.11, 2.04, and 1.96 for the acetyl groups; *ii*) the HMBC correlation of a typically *O*-deshielded signal at δ(C) 81.92 (*s*, C(11)) with H–C(13), Me(17) and H–C(10) and of δ(C) 71.46 (*d*, C(12)) with the typically acyl-deshielded proton H–C(13) at δ(H) 5.68; *iii*) a signal for another acyl-deshielded proton at δ(H) 5.17, coupled with H–C(13) and heterocorrelated with δ(C) 69.38 (*d*); *iv*) resonances for an isopropyl group that, taking into account the signals δ(H) 5.17 and 1.81 for two nearby coupled protons, must be positioned at C(14); *v*) resonances for two methine groups at δ(H) 2.27 and 3.38 coupled with δ(C) 43.43 (*d*) and 46.14 (*d*), respectively, and nearby coupled with, and attributed to, H–C(1) and H–C(10) from HMBC experiments. A trisubstituted exocyclic C=C bond is suggested both by *s*'s at δ(H) 5.01 and 5.51 (coupled with δ(C) 115.57 (*t*), which is further HMBC correlated with protons at C(5) and C(8)) and, finally, by a *s* at δ(C) 145.41, which shows heterocorrelation with H_b–C(16) and the protons at C(5), C(8), and C(9).

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[1]) Viewing sarcodictyins, eleutherobin, eleuthosides, and valdivones as a single structural group (eleuthesides) [14a] would obscure the unique conjugative reactivity of sarcodictyins at the carboxylate-bearing olefinic group [10]. This property seems to have escaped the attention of other authors, too [14b], who implicitly and unwarrantedly attributed such conjugative aptitude also to eleutherobin in an attempt to assess the causes of non-functional stabilization of tubulin (a phenomenon that, for these compounds, was first described for sarcodictyins [14c] and later for eleutherobin [11]).

Table. *NMR Spectral Data for Massileunicellin A* (**7**) *in* $CDCl_3$

	δ (H)[a])	δ (C)	NOE[b])	HMBC[c])
H–C(1)	2.27 (*dd*, *J*(1,14) = 11.8, *J*(1,10) = 7.8)	43.43 (*d*)	H(10), H(13)	C(9), C(10), C(14), C(15), C(18)
H–C(2)	3.56 (*s*)	91.53 (*d*)	H–C(14), Me(15), H–C(18)	C(1), C(3), C(9), C(14)
C(3)	–	87.59 (*s*)		
CH_2(4)	2.15 (*m*); 1.87 (*m*)	36.40 (*t*)		
CH_2(5)	2.05 (*m*)			
		37.33 (*t*)		
	2.90 (*m*)			C(6), C(7), C(16)
C(6)	–	107.15 (*s*)		
C(7)	–	145.41 (*s*)		
CH_2(8)	3.16 (*dd*, J_{gem} = 16.3, *J*(8α,9) = 3.2, H_α)	42.19 (*t*)	H–C(9), H_a–C(16)	C(7), C(10), C(16)
	2.44 (*dd*, J_{gem} = 16.3, *J*(8β,9) = 3.2, H_β)		H–C(9), H_a–C(16), Me(17)	C(6), C(7), C(16)
H–C(9)	4.53 (*dt*, *J*(9,10) = 8.9, *J*(9,8α) ~ *J*(9,8β) = 3.2)	79.05 (*d*)	H_α–C(8), Me(17)	C(7)
H–C(10)	3.38 (*dd*, *J*(10,9) = 8.9, *J*(10,1) = 7.8)	46.14 (*d*)	H–C(1), Me(17)	C(1), C(9), C(11), C(12), C(14)
C(11)	–	81.92 (*s*)		
H–C(12)	5.68 (*d*, *J*(12,13) = 2.1)	71.46 (*d*)	H–C(13)	C(10), C(11), C(13), C(14), CH_3CO
H–C(13)	5.17 (*dd*, *J*(13,14) = 11.8, *J*(13,12) = 2.1)	69.38 (*d*)	Me(20)	CH_3CO
H–(14)	1.81 (br. *t*, *J*(14,13) ~ *J*(14,1) = 11.8)	39.63 (*d*)		
Me(15)	1.26 (*s*)	21.15 (*q*)	H–C(1), H–C(2)	C(2), C(3), C(4)
CH_2(16)	5.01 (*s*, H_a);	115.57 (*t*)	H_β–C(8), H_b–C(16)	C(6), C(8)
	5.51 (*s*, H_b)		H_a(16)	C(6), C(7), C(8)
Me(17)	1.52 (*s*)	24.56 (*q*)	H–C(9), H–C(10), H–C(12)	C(10), C(11), C(12)
H–C(18)	1.64 (*m*)	29.58 (*d*)		
Me(19)	0.82 (*d*, *J*(19,18) = 6.9)	15.56 (*q*)		C(14), C(18), C(20)
Me(20)	1.01 (*d*, *J*(20,18) = 6.9)	23.87 (*q*)		C(14), C(18), C(19)
OH	2.62 (*s*)	–		C(5)
AcO	2.11, 2.04, 1.96 (3 *s*)	169.93, 169.50, 169.33 (3 *s*), 22.41, 20.97, 20.39 (3 *q*)		

[a]) δ in ppm; *J* in Hz.
[b]) NOE Enhancement observed for the indicated H-atom(s) by irradiation of the proton(s) listed in the same row.
[c]) Heterocorrelation of the indicated C-atom(s) with the proton(s) listed in the same row.

The *s* at δ(C) 107.15 (which shows heterocorrelation with H_β–C(8), a proton at C(5), and both protons at C(16)) can be assigned to the hemiketal center C(6)[2]). Signals for two O-deshielded methine groups (δ(H) 3.56 and 4.53, coupled with δ(C) 91.53 (*d*) and 79.05 (*d*), respectively), for a quaternary center at δ(C) 87.59 and for three *t* at δ(C) 36.40, 37.33, and 42.19 (the latter directly correlated with a *dd* at δ(H) 3.16 and 2.44, both showing further coupling with H–C(9)), support a ten-membered cycle fused with a six-membered ring and bearing 2,9- and 3,6-O-bridges, with a hemiketal center at C(6).

The relative configuration at the six-membered ring of **7** (including the *cis* ring fusion at C(1),C(10) and the β-position of the isopropyl group at C(14), as well as the α-position of Me(17)), the *cis* relationship of the protons at C(2) and C(9), and the β-Me(15) rest on NOE data (*Table*). The low value of the interproton coupling constant $J(12,13) = 2.1$, and a NOE enhancement observed between signals at δ(H) 5.68 and 5.17, point to *cis*-related AcO at C(12) and C(13).

MS and NMR data (*Exper. Part*) show that massileunicellin B (**9**) is isomeric with **7**. Partial transformation of **9** into the methyl ketal **10** occurred either during some days in $CDCl_3$ solution in the presence of MeOH[3]), or immediately on evaporation to dryness of a $CDCl_3$/MeOH solution. Complete transformation of compound **9** into **10** could be achieved on repeating the above procedures or on treatment of fresh **9** in MeOH with PPTS which yielded **10** as a single stereoisomer. The corresponding transformations of compound **7** into **8** and compound **11** into **12** could be similarly obtained (*Scheme*).

NMR and MS data (*Exper. Part*) show a structural similarity of massileunicellin C (**11**) with both **7** and **9**, in particular as to both the hemiketal moiety and the substitution pattern at the six-membered ring.

The trisubstituted endocyclic C(7)=C(8) bond of **9** finds evidence in a *dq* at δ(H) 5.50, which is correlated with δ(C) 128.38 (*d*), δ(C) 142.48 (*s*), and δ(H) 1.83 (*d*); the latter is further correlated with δ(C) 23.70 (*q*), attributable to Me(16). (*Z*)-Configuration at C(7)=C(8) rests on NOE enhancement between H–C(8) and Me(16). The presence of a Me group at C(7) of **11** is suggested by a *d* at δ(H) 0.96 correlated with δ(C) 18.40 (*q*, Me(16)) and δ(H) 2.33 (*ddq*, correlated with δ(C) 37.71 (*d*, H–C(7)), in agreement with the composition $C_{26}H_{40}O_9$ (HR-EI-MS) for ketal derivative **12**. The α-position for Me(16) rests on a NOE enhancement at H–C(7) on irradiating H–C(10), and is corroborated by a NOE enhancement between $H_{\beta eq}$–C(8) and H–C(9).

The least-strain conformations for massileunicellin A (**7**) and C (**11**), derived from molecular-mechanics (MM) calculations (*Fig. 2*), nicely reproduce the experimental coupling constants *J* and interproton distances (*Table* and *Exper. Part*). It can be seen that the two bridge O-atoms lie on opposite sides, while Me(20) points toward H–C(13). The latter feature finds evidence for massileunicellin A (**7**) in NOE enhancements at Me(20) on irradiating H–C(13) and at H–C(18) on irradiating H–C(2) (*Table*). Correspondingly, NOE enhancements were observed at H–C(13) and H–C(1) of ketal **12** on irradiating Me(20) (*Exper. Part*).

Although C(16) prefers the β-orientation in the least-strained conformation of massileunicellin A (**7**) (*Fig. 2*), rapid flipping to the α-position occurs on the NMR time

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[2]) A series of minor (*ca.* 7%) signals (^{1}H-NMR: 5.62 (*d*, $J = 2.1$); 5.40 (br. *s*); 5.30 (*m*); 3.61 (*s*); ^{13}C-NMR: 119.48 (*t*); 90.11 (*d*); 78.29 (*d*); 73.19 (*s*); 72.19 (*d*); 69.63 (*d*); 43.38 (*d*); 42.47 (*t*); 39.08 (*d*); 28.52 (*d*); 23.96 (*q*); 22.56 (*q*); 15.20 (*q*)) might result from 3,6-bridge-opened form (*i.e.*, having C(6)=O and C(3)–OH) in equilibrium with **7**, under the reasonable expectation that all other NMR signals for the two forms are superimposable. Consistently, on treatment with pyridinium *p*-toluenesulfonate (PPTS) in MeOH, both the hemiketal and the presumptive keto forms disappeared to give **8** only.

[3]) Because of these partial transformations, it is safer to report polarimetric data of the pure ketals **10** and **12** rather than of the natural products. Compound **7** proved stable in $CDCl_3$ in the absence of MeOH.

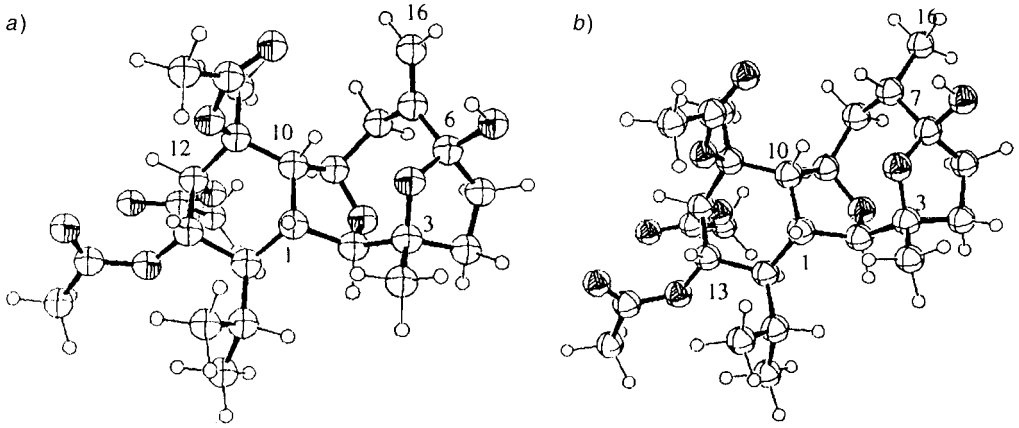

Fig. 2. *Energy-minimized conformations of* a) *massileunicellin A* (**7**) *and* b) *massileunicellin C* (**11**) *as derived from molecular-mechanics calculations in accordance with NMR data*

scale, as showed by NOE enhancements at both H–C(9) and H_a–C(16) on irradiating either proton at C(8). Correspondingly, MM calculations resulted also in a minor conformer with α-orientation of C(16) of slightly higher strain energy (not shown in *Fig. 2*).

Relative configurations for massileunicellins A–C (**7**, **9**, and **11**, resp.) and their methyl derivatives **8**, **10**, and **12** at C(1), C(2), C(9), C(10), and C(14) are the same as in all other cladiellane and eunicellane diterpenes [8a]. Separately, and when relevant, this is also true for the configurations at C(11), C(12), and C(13) [1b][2][3b–g][4a][7]. Few eunicellane diterpenes carrying substituents at all these three centers are known [1b][3g][4a], and none of them has the relative configurations of massileunicellins A–C (**7**, **9**, and **11**, resp.).

The configurations at C(3) and C(6) are consistent with nucleophilic attack at C(6)=O by a β-oriented OH–C(3), which is the typical orientation at C(3), for either OH [3b–d,f] [8c] or the more common ester substituents [2][3c,e,g][4a,b][6][8b].

Where determined (eunicellin dibromide [2], 3-acetoxy-2,12-bis(butanoyloxy)cladiellin-8-ene-4,11-diol [3g], palmonine F [6], and sclerophytin C [8a]), chiral centers C(9), C(2), C(3), C(9), C(10), and C(14) in eunicellane diterpenes have (*R*) configuration, in a correlation not affected by the *Cahn-Ingold-Prelog* priority of the actual substituents. The same may thus be expected for massileunicellins, but our attempts at preparing a 12,13-dibenzoyl derivative of 6-*O*-methylmassileunicellin C (**12**) to check the point *via* dichroic analysis failed.

3. Perspective. – It is striking that the sole diterpenoid – a classical eunicellane diterpene of type **2** – reportedly isolated from *Eunicella cavolinii* from the Bay of Naples [15b] is absent from our sample of unmistakably this gorgonian species from the Marseille area. Voucher material of our gorgonian was deposited at the 'Senckenberg Museum', Frankfurt (SMF 6998), whereas, unfortunately, no voucher specimen of the Bay of Naples material was made available [15]. By the same vein it is surprising that the eunicellane diterpenes **7**, **9**, and **11** described here were not reported for the Bay of

Naples organism. Both the latter [15a] and our gorgonian contain huge amounts of 11α-hydroxy-pregna-4,20-dien-3-one acetate.

Good morphological descriptions of *E. cavolinii* (KOCH, 1887) (Cnidaria, Anthozoa, Gorgoniidae) are available [16]. This gorgonian is wide-spread in the Mediterranean, where its highly branched fan-shaped colonies, generally orange in color (or other yellow nuances), are easily observed in diving depths on steep cliffs. It is devoid of zooxanthellae, whereas the often co-occurring Mediterranean congener *Eunicella singularis* (ESPER, 1791) has such symbionts. Thus, at this stage it is difficult even to relate the terpenoid differences to associated microbial populations in the gorgonian from the different areas. Should the identification of the Bay of Naples gorgonian referred to as *E. cavolinii*, with the described array of products [15], be confirmed, we would be faced with an unusual problem of possibly seasonal dependence or intraspecific geographic diversification for gorgonians. This would call for genome and allozyme analysis, at least the former of which seems to have been carried out for only tropical gorgonians from coral reefs [17].

Therapeutically oriented bioassays with the products from our gorgonian were disappointing, no significant cytotoxic or antiviral activity resulting from *in vitro* assays of these terpenoids on human tumor cells (KB and doxorubicin-resistant L1210) and *Dengue* virus, while 11α-hydroxy-pregna-4,20-dien-3-one showed only marginal cytotoxicity on KB cells (77% at 10 μg ml^{-1}). In view of reportedly high cytotoxicity on various tumor cells by several eunicellane diterpenes [6][7], lack of cytotoxicity of massileunicellins on KB and L1210 cells may be surprising. A cross check came at hand with labiatin B [7] and palmonine D [6], reisolated from another gorgonian in our laboratories. Labiatin B was previously reported to show cytotoxicity on human colon cancer cells HCT-116 with an ED_{50} of 0.85 μg ml^{-1} [7], and palmonine D to exhibit cytotoxicity on P388 and MEL28 tumor cells with an ED_{50} of 5 μg ml^{-1} [6]. In our laboratories, labiatin B was assayed on both KB and doxorubicin-resistant L1210 cell lines, while palmonine D was only assayed on KB cells, and all resulted inactive. Therefore, either there is an interesting selectivity of these terpenoids for different tumor cells or, possibly, sensitive tumor cells have been used in previous assays [6][7], leading to illusory results.

It is worth mentioning that, in our experimentation, neither labiatin B nor palmonine D showed any effect on calf tubulin, in contrast with the strong induction of nonfunctional polymerization of tubulin by sarcodictyins [14c] (which compete for the binding site of paclitaxel [18]), sarcodictyin analogues made available in one of the first solid- and solution-phase libraries based on natural products [19], and by eleutherobin [14e].

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Experimental Part

General. All evaporations were carried out at r.t. under reduced pressure. Flash chromatography (FC): *Merck Si-60* (15–25 μm) and *Merck* reversed-phase *RP-18* (15–25 μm). TLC: *Merck silica gel 60 PF_{254}* and *Merck RP-18 F_{254}*. HPLC: *Merck LiChrosorb CN* (7 μm) and reversed-phase *LiChrospher RP18* (7 μm), 25 × 1 cm columns, under UV monitoring at 215 nm, solvent flow 5 ml min^{-1}. Polarimetric data: *Jasco-DP-181* polarimeter, $[\alpha]_D$ values in 10^{-1} deg ml g^{-1}. NMR: *Varian-XL-300*, ^{1}H at 299.94 MHz and ^{13}C at 75.43 MHz in

$CDCl_3$; δ in ppm rel. to internal $SiMe_4$ (= 0 ppm); multiplicities from DEPT; $^1H,^1H$ correlations from COSY60 and selective decoupling irradiations; $^1H,^{13}C$ assignments from one-bond and long-range $^1H,^{13}C$-COSY, or $^{13}C,^1H$-NMR by inverse detection shift correlation experiments; NOE stands for differential NOE 1D data, reported as 'irradiated proton → NOE observed on the proton(s)'; arbitrary atom numbering. EI-MS, FAB-MS, and EI-HR-MS: *Kratos-MS80* with home-built computerized data-acquisition system and *Vacumetrics-DIP* gun for FAB; *m/z* (rel. %). Molecular-mechanics calculations: programs PCMODEL 4.0 from *Serena Software*, Bloomington, Indiana, and MM3(96) from QCPE, Indiana University.

Collection and Isolations. Eunicella cavolinii colonies were collected at end of July, 1997, from a steep cliff, depth 10–25 m, at Grand Conglu island near Marseille, NW Mediterranean, France. The colonies were carefully cut off, leaving the bases with a few cm of stem on the rock for regeneration. The dry weight of the remaining extracted coenenchyme and 'horny' axis was 535 g. Immediately after collection, the gorgonian was soaked into 95% EtOH (sample 852M) and extracted leaving, after evaporation, 18 g of residue that was subjected to FC (*Si-60*, hexane/AcOEt/MeOH gradient elution, 22 fractions of 50 ml each). *Frs. 9–12* were subjected to FC (*RP-18*, H_2O/MeCN gradient elution, 18 fractions). *Frs. 13–15* of the latter contained the known 11α-hydroxypregna-4,20-dien-3-one acetate (158 mg). *Fr. 9* was subjected to HPLC (*CN*, hexane/i-PrOH 85:15) to give pure **9** (t_R 7.5 min, 1.9 mg, 0.010% of raw extract). *Fr. 13* from the FC on Si-60 was subjected to FC (*RP-18*) as above, and the resulting *Frs. 6–7* were combined and subjected to HPLC (*RP-18*, MeOH/H_2O 7:3), to give pure **7** (t_R 7.0 min; 6.2 mg, 0.034%) and pure **11** (t_R 8.5 min; 3.2 mg, 0.017%).

Massileunicellin A (= *(1S*,2S*,3S*,4R*,4aR*,5R*,6R*,9R*,12R*,12aS*)-Tetradecahydro-1,6-dimethyl-10-methylidene-4-(1-methylethyl)-5,12 : 6,9-diepoxybenzocyclododecene-1,2,3,9-tetrol 1,2,3-Triacetate*; **7**). White amorphous powder. $[\alpha]_D^{20} = -5$ ($c = 0.3$, $CHCl_3$). EI-MS: 479 (0.2, $[M-CH_3]^+$), 477 (0.2, $[M-OH]^+$), 476 (0.3, $[M-H_2O]^{+\cdot}$), 434 (1.5, $[M-AcOH]^{+\cdot}$), 374 (2.3, $[434-AcOH]^{+\cdot}$), 314(4), 99(22), 43(100). HR-EI-MS: 476.2405 ± 0.0030 ($C_{26}H_{36}O_8^{+\cdot}$; calc. 476.2410), 434.2299 ± 0.0030 ($C_{24}H_{34}O_7^{+\cdot}$; calc. 434.2304). FAB-MS (3-nitrobenzyl alcohol): 495 (1, $[M+H]^+$).

Massileunicellin B (= *(1S*,2S*,3S*,4R*,4aR*,5R*,6R*,9R*,10Z,12R*,12aS*)-1,2,3,4,4a,5,6,7,8,9,12,12a-Dodecahydro-1,6,10-trimethyl-4-(1-methylethyl)-5,12 : 6,9-diepoxybenzocyclodecene-1,2,3,9-tetrol 1,2,3-Triacetate*; **9**). White amorphous solid: ^{1}H-NMR: 2.28 (*dd*, *J*(1,14) = 11.8, *J*(1,10) = 7.8, H–C(1)); 3.71(*s*, H–C(2)); 2.00–2.15 (*m*, 2H–C(4), H_a–C(5)); 2.60 (*m*, H_b–C(5)); 5.50 (*dq*, *J*(8,9) = 6.2, *J*(8,16) = 1.4, H–C(8)); 4.67 (*dd*, *J*(9,10) = 8.7, *J*(9,8) = 6.2, H–C(9)); 3.45 (*dd*, *J*(10,9) = 8.7, *J*(10,1) = 7.8, H–C(10)); 5.76 (*d*, *J*(12,13) = 2.8, H–C(12)); 5.15 (*dd*, *J*(13,14) = 12.0, *J*(13,12) = 2.8, H–C(13)); 1.81 (*m*, H–C(14)); 1.30 (*s*, Me(15)); 1.83 (*d*, *J*(16,8) = 1.4, Me(16)); 1.55 (*s*, Me(17)); 1.64 (*m*, H–C(18)); 0.84 (*d*, *J*(19,18) = 6.9, Me(19)); 1.02 (*d*, *J*(20,18) = 6.9, Me(20)); 2.11, 2.04, 1.96 (3 *s*, 3 MeCO). ^{13}C-NMR: 42.25 (*d*, C(1)); 91.87 (*d*, C(2)); 86.41 (*s*, C(3)); 39.06 (*t*, C(4)); 42.00 (*t*, C(5)); 108.99 (*s*, C(6)); 142.48 (*s*, C(7)); 128.38 (*d*, C(8)); 77.33 (*d*, C(9)); 54.86 (*d*, C(10)); 81.82 (s, C(11)); 71.15 (*d*, C(12)); 69.51 (*d*, C(13)); 36.46 (*d*, C(14)); 21.12 (*q*, C(15)); 23.70 (*q*, C(16)); 24.37 (*q*, C(17)); 29.64 (*d*, C(18)); 15.80 (*q*, C(19)); 23.87 (*q*, C(20)); 20.88, 22.11, 22.37 (3 *q*, *Me*CO)); 169.99, 169.54,169.33 (3 *s*, Me*C*O). NOE: 4.67 → H–C(8), Me(17); 5.15 → H–C(12), Me(20); 5.76 → H–C(13), Me(20); 5.57 → H–C(9), Me(16); 1.30 → H–C(1), H–C(2). EI-MS: 479(0.8), 477(1.8), 476(1.3), 434(6), 374(2), 314(17), 99(27), 43(100). FAB-MS (3-nitrobenzyl alcohol): 495 (0.5, $[M+H]^+$).

Massileunicellin C (= *(1S*,2S*,3S*,4R*,4aR*,5R*,6R*,9R*,10S*,12R*,12aS*)-Tetradecahydro-1,6,10-trimethyl-4-(1-methylethyl)-5,12 : 6,9-diepoxybenzocyclododecene-1,2,3,9-tetrol 1,2,3-Triacetate*; **11**). White amorphous solid. ^{1}H-NMR ($CDCl_3$): 2.23 (*dd*, *J*(1,14) = 11.8, *J*(1,10) = 7.8, H–C(1)); 3.54 (*s*, H–C(2)); 1.90–2.15 (*m*, 2H–C(4)); 1.75, 2.56 (2 *m*, 2H–C(5)); 2.33 (*ddq*, *J*(7,8$_{\alpha ax}$) = 11.2, *J*(7,8$_{\beta eq}$) = 5.2, *J*(7,16) = 6.8, H$_{\beta ax}$–C(7)); 1.74 (*ddd*, J_{gem} = 16.0, *J*(8$_{\beta eq}$, 9) = 3.2, *J*(8$_{\beta eq}$,7) = 5.2, H$_{\beta eq}$–C(8)); 1.97 (*ddd*, J_{gem} = 16.0, *J*(8$_{\alpha ax}$,7) = 11.2, *J*(8$_{\alpha ax}$,9) = 3.2, H$_{\alpha ax}$–C(8)); 4.40 (*dt*, *J*(9,10) = 9.0, *J*(9,8$_{\alpha ax}$) ≈ *J*(9,8$_{\beta eq}$) = 3.2, H–C(9)); 3.82 (*t*, *J*(10,9) ~ *J*(10,1) = 9.0, H–C(10)); 5.48 (br.*d*, *J*(12,13) = 2.8, H–C(12)); 5.21 (*dd*, *J*(13,14) = 11.8, *J*(13,12) = 2.8, H–C(13)); 1.82 (*m*, H–C(14)); 1.26 (*s*, Me(15)); 0.96 (*d*, *J*(16,7) = 6.8, Me(16)); 1.51 (*s*, Me(17)); 1.61 (*m*, H–C(18)); 0.83 (*d*, *J*(19,18) = 7.0, Me(19)); 1.01 (*d*, *J*(20,18) = 7.0, Me(20)); 2.09, 2.06, 1.96 (3 *s*, 3 MeCO). ^{13}C-NMR: 43.36 (*d*, C(1)); 91.23 (*d*, C(2)); 86.48 (*s*, C(3)); 32.42 (*t*, C(4)); 36.06 (*t*, C(5)); 111.57 (*s*, C(6)); 37.71 (*d*, C(7)); 41.24 (*t*, C(8)); 79.00 (*d*, C(9)); 44.03 (*d*, C(10)); 82.11(*s*, C(11)); 72.15 (*d*, C(12)); 69.55 (*d*, C(13)); 39.33 (*d*, C(14)); 21.15 (*q*, C(15)); 18.40 (*q*, C(16)); 24.80 (*q*, C(17)); 29.59 (*d*, C(18)); 15.60 (*q*, C(19)); 23.86 (*q*, C(20)); 22.57, 21.16, 20.94 (3 *q*, 3 *Me*CO); 169.97, 169.67, 169.57 (3 *s*, Me*C*O). EI-MS: 496 (0.2, $M^{+\cdot}$), 479(1), 478 (2, $[M-H_2O]^+$), 436(1), 376(1), 358(1), 316(2), 99(25), 43(100). HR-EI-MS: 478.2564 ± 0.0030 ($C_{26}H_{38}O_8^{+\cdot}$; calc. 478.2566).

Methylation of Massileunicellins A (**7**)*, B* (**9**)*, and C* (**11**). To a soln. of **7** (3.0 mg, 0.006 mmol) in MeOH (1 ml), cat. amounts of pyridinium *p*-toluenesulfonate (PPTS) were added. The mixture was stirred at r.t. until all **7** had disappeared (15 min, TLC monitoring), and was then evaporated. The residue was passed through a *Si-*

LiChrolut column (*Merck*) with hexane/AcOEt 6:4, to give pure **8** (2.9 mg, 94%). Similarly, compounds **9** and **11** gave pure **10** and **12**, resp.

6-O-Methylmassileunicellin A (=*(1S*,2S*,3S*,4R*,4aR*,5R*,6R*,9R*,12R*,12aS*)-Tetradecahydro-9-methoxy-1,6-dimethyl-10-methylene-4-(1-methylethyl)-5,12 : 6,9-diepoxybenzocyclododecene-1,2,3-triol Triacetate*; **8**). ^{1}H-NMR: 2.30 (*dd*, *J* = 11.8, 7.8, H–C(1)); 3.57 (*s*, H–C(2)); 2.15–1.90 (*m*, 2 H–C(4), H–C(5)); 2.80 (*m*, H–C(5)); 3.20, 2.46 (2 *m*, 2 H–C(8)); 4.53 (*dt*, *J* = 8.9, 3.2, H–C(9)); 3.38 (*m*, H–C(10)); 5.84 (*d*, *J* = 2.3, H–C(12)); 5.14 (*dd*, *J* = 11.8, 2.4, H–C(13)); 1.81 (*m*, H–C(14)); 1.26 (*s*, Me(15)); 5.00, 5.65 (2 *s*, 2 H–C(16)); 1.52 (*s*, Me(17)); 1.60 (*m*, H–C(18); 0.82 (*d*, *J* = 6.9, Me(19)); 1.01 (*d*, *J* = 6.9, Me(20)); 2.11, 2.04, 1.96 (3 *s*, 3 *Me*CO); 3.34 (*s*, MeO). EI-MS: 508 (2, $M^{+\cdot}$), 477 (1), 448 (2), 388 (1), 328 (2), 99 (27), 43 (100). HR-EI-MS: 508.2667 ± 0.0030 ($C_{27}H_{40}O_9^{+\cdot}$; calc. 508.2670).

6-O-Methylmassileunicellin B (=*(1S*,2S*,3S*,4R*,4aR*,5R*,6R*,9R*,10Z,12R*,12aS*)-1,2,3,4,4a,5,6,7,8,9,12,12a-Dodecahydro-9-methoxy-1,6,10-trimethyl-4-(1-methylethyl)-5,12 : 6,9-diepoxybenzocyclododecene-1,2,3-triol Triacetate*; **10**). $[\alpha]_D^{20} = -15$ (*c* = 0.1, MeOH). ^{1}H-NMR: 2.30 (*dd*, *J* = 11.8, 7.8, H–C(1)); 3.71 (*s*, H–C(2)); 2.00–2.15 (*m*, 2 H–C(4), H–C(5)); 2.48 (*m*, H–C(5)); 5.56 (*dq*, *J* = 6.3, 1.4, H–C(8)); 4.67 (*dd*, *J* = 8.7, 6.3, H–C(9)); 3.72 (*m*, H–C(10)); 5.80 (*d*, *J* = 2.7, H–C(12)); 5.14 (*dd*, *J* = 12.0, 2.7, H–C(13)); 1.80 (*m*, H–C(14)); 1.31 (*s*, Me(15)); 1.75 (*d*, *J* = 1.4, Me(16)); 1.54 (s, Me(17)); 1.64 (*m*, H–C(18)); 0.85 (*d*, *J* = 6.9, Me(19)); 1.02 (*d*, *J* = 6.9, Me(20)); 2.11, 2.05, 1.96 (3 *s*, 3 *Me*CO); 3.23 (*s*, MeO). ^{13}C-NMR: 169.99 (*s*), 169.56 (*s*); 169.34 (*s*); 142.70 (*s*); 129.65 (*d*); 112.36 (*s*); 91.90 (*d*); 86.26 (*s*); 81.80 (*s*); 71.09 (*d*); 69.57 (*d*); 55.03 (*d* or *q*); 49.67 (*q* or *d*); 42.05 (*d*); 39.11 (*t*); 36.75 (*d*); 29.58 (*d*); 26.32 (*q*); 23.73 (*q*); 23.43 (*q*); 22.40 (*q*); 21.80 (*q*); 21.14 (*q*); 21.06 (*q*); 20.83 (*q*); 15.83 (*q*). EI-MS: 508 (1.6, $M^{+\cdot}$), 448 (3), 388 (2), 328 (2), 99 (23), 43 (100). FAB-MS (3-nitrobenzyl alcohol): 509 (0.6, $[M+H]^+$).

6-O-Methylmassileunicellin C (=*(1S*,2S*,3S*,4R*,4aR*,5R*,6R*,9R*,12R*,12aS*)-Tetradecahydro-9-methoxy-1,6,10-trimethyl-4-(1-methylethyl)-5,12 : 6,9-diepoxybenzenecyclododecene-1,2,3-triol Triacetate*; **12**). $[\alpha]_D^{20} = +12$ (*c* = 0.3, MeOH). ^{1}H-NMR: 2.29 (*dd*, *J* = 11.8, 7.8, H–C(1)); 3.54 (*s*, H–C(2)); 1.85–2.10 (*m*, 2 H–C(4), H–C(5)); 2.46 (*m*, H–C(5), H–C(7)); 1.76 (*ddd*, *J* = 15.8, 5.2, 3.2, $H_{\beta eq}$–C(8)); 1.94 (*ddd*, *J* = 15.8, 11.2, 3.2, $H_{\alpha ax}$–C(8)); 4.43 (*dt*, *J* = 9.0, 3.2, H–C(9)); 3.69 (*t*, *J* = 9.0, H–C(10)); 5.60 (br. *d*, *J* = 2.6, H–C(12)); 5.18 (*dd*, *J* = 11.8, 2.6, H–C(13)); 1.80 (*m*, H–C(14)); 1.26 (*s*, Me(15)); 0.88 (*d*, *J* = 6.8, Me(16)); 1.51 (*s*, Me(17)); 1.61 (*m*, H–C(18)); 0.84 (*d*, *J* = 7.0, Me(19)); 1.01 (*d*, *J* = 7.0, Me(20)); 2.09, 2.07, 1.96 (3 *s*, 3 *Me*CO); 3.23 (*s*, MeO). NOE: 4.43 → H_{eq}–C(8), Me(17); 3.69 → H–C(1), H–C(7); 5.60 → H–C(13), Me(17); 5.18 → H–C(12); 1.26 → H–C(1), H–C(2); 0.88 → H–C(7), MeO–C(9); 1.51 → H–C(9), H–C(10), H–C(12); 0.82 → H–C(1), H–C(13); 3.23 → H_{eq}–C(8). EI-MS: 510 (0.8, $M^{+\cdot}$), 479 (1), 450 (1), 419 (1), 390 (1), 359 (2), 43 (100). HR-EI-MS: 510.2821 ± 0.0030 ($C_{27}H_{42}O_9^{+\cdot}$; calc. 510.2829).

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