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Callipeltosides B and C, Two Novel Cytotoxic Glycoside Macrolides from a Marine Lithistida Sponge *Callipelta* sp.

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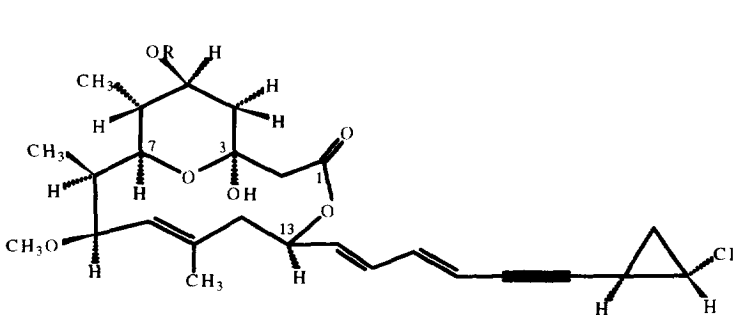
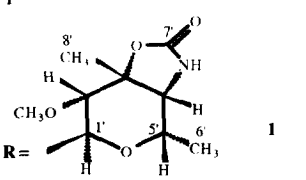
Abstract: Following the characterization of callipeltoside A (**1**), the first member of a novel class of marine glycoside macrolides, two more bioactive constituents, callipeltoside B (**2**) and C(**3**), were isolated from *Callipelta* sp. in very low amounts. The structures, assigned on the basis of spectral analysis, include the same 14-membered macrolide as in callipeltoside A (**1**) but differed in the saccharide moieties. © 1997 Elsevier Science Ltd. All rights reserved.

Marine sponges of the order Lithistida continue to be exceptionally rich source of structurally unique and biologically active macrolides[1] and peptides.[2] In the course of our search for new antitumor and antiviral substances from marine sponges, we examined the extracts of the lithistid sponge *Callipelta* sp., which exhibited marked activity in cytotoxic assays against KB and P388 cells and in the anti-HIV tests. We isolated from the dichloromethane-methanol extract the major cytotoxic constituents, callipeltins A-C, and assigned to them a peptidal structure.[3,4] Callipeltin A was found to protect *in vitro* cells infected by HIV virus.[3] Further investigations on the dichloromethane extract from several collections of this sponge (2.5 Kg, freeze-dried in total) afforded in low yield a novel cytotoxic glycoside macrolide, callipeltoside A, whose structure **1** was assigned by interpetration of spectral data.[5] We now report the isolation and the structural elucidation of two further very minor metabolites, callipeltosides B-C (**2-3**), which are related to callipeltoside A.





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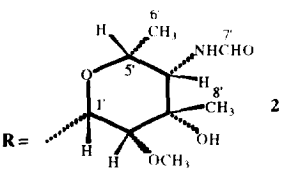

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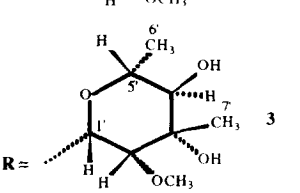

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The freeze-dried sponge specimens, collected several times in 1992-1994 off the east coast of New Caledonia at a depth of 5-10 m, were sequentially extracted with *n*-hexane, dichloromethane and 8:2 dichloromethane-methanol. The CH_2Cl_2 fraction was 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 B (**2**, 1.0 mg) and callipeltoside C (**3**, 0.8 mg) along with callipeltoside A (**1**, 3.5 mg).

The ESMS of callipeltoside B (**2**) gave pseudomolecolar ions at *m/z* 702.36-704.34 [$(M+Na)^+$, 100, 36], two mass unit higher than callipeltoside A (**1**), corresponding to a molecular formula $C_{35}H_{50}O_{10}NCl$. Analysis of

Table 1. NMR data of callipeltoside B (**2**) in $CDCl_3$ (500 MHz)

Position	δH	δC	HMBC	ROESY[a]
1	-	171.7		
2	2.53 d (13.5), 2.43 d (13.5)	44.7	C1, C3	5.04
3	-	95.2		
4	2.21 dd (11.2, 4.4) 1.31 dt (11.2, 2.0)	42.3	C3, C5, C6	5.04, 3.71
5	3.71 dt (11.7, 4.4)	77.5	C1'	0.94, 4.97
6	1.46 m	38.3		
7	3.64 dd (10.5, 2.0)	74.8		0.94, 2.20, 5.04, 4.97
8	2.20 m	36.8	C9, C6	3.23
9	3.81 dd (9.1, 1.7)	79.6	C24, C11	3.23, 1.74, 0.98
10	5.29 br dd (9.1)	127.7	C9, C12, C23	3.23, 0.98, 2.31
11	-	132.6		
12	2.31 dd overlapped 2.09 dd overlapped	46.9	C10, C23	
13	5.82 m	71.6	C12	1.74
14	5.76 dd (15.3, 7.1)	132.7	C13, C16	6.48
15	6.26 dd (15.3, 10.8)	131.0	C13	5.57
16	6.48 dd (15.7, 10.8)	140.0	C14, C18	
17	5.57 dd (15.7, 1.7)	112.6	C15, C19	
18	-	78.3		
19	-	92.6		
20	1.80 m overlapped	34.0		
21	1.29 m	19.3	C19	
22	3.18 m	55.4		1.29
23	1.73 s	16.2		
24	0.98 d (6.9)	6.5	C7, C9	
25	0.94 d (6.4)	12.4	C6, C7	3.52
OCH_3	3.23 s	55.2	C9	
3-OH	5.03 d (2.0)	-		
1'	4.97 br s	99.2	C5, C5', C2', C3'	2.97, 4.11, 3.97, 1.44
2'	2.97 br s	83.6	C1', C3', C4', C8'	1.44
3'	-	68.1		
4'	3.97 d (10.5)	55.6	C3', C7', C5'	1.44, 6.32, 8.37, 4.11
5'	4.11 q (6.5)	64.9	C1'	1.44
6'	1.20 d (6.5), 1.22 d (6.5)	17.4	C4', C5'	6.32
7'	8.36 br s, 7.92 d (10.5)	151.4	C4'	
8'	1.44 s, 1.42 s	23.8	C3', C2', C4'	
OCH_3	3.47 s, 3.43 s	59.3	C2'	
NH	6.32 d (10.5), 6.13 t (10.5)	-		

[a]Roesy mixing time t_m=500 ms

the proton and carbon NMR data with the aid of COSY, HMBC and ROESY connectivities (Table 1) indicated

that **2** possessed the same macrolide portion, including stereochemistry, as **1**. ^{1}H NMR spectrum of the sugar portion exhibited doubled signals in a ratio 8:2, indicating the existence of two inseparable conformers or isomers. The ^{1}H NMR spectrum indicated two sets of signals for a formyl proton at δ 8.36 br s (major) and 7.92 d (J=10.5 Hz) (minor), which correlated by COSY with an exchangeable NH broad doublet signal at δ 6.32 (J=10.5 Hz) and with an exchangeable NH triplet signal (J=10.5 Hz), respectively. In the COSY spectrum, the NH protons were correlated to a methine at δ 3.97 (d, J=10.5 Hz). The presence of a formamide carbonyl was also evident from the ^{13}C NMR signal at δ 151.4 and its connections were supported by HMBC correlations of NH (δ 6.30 and 6.13) and CH (δ 3.97) to carbonyl group (δ 151.4). Thus, we have assumed that a restricted rotation about the NH formyl group was responsible for the observed doubled signals for NH, CHO, CH_3-6' (1.20 and 1.22), CH_3-8' (δ 1.44 and 1.42), and OCH_3 (δ 3.47 and 3.43), all of them in a 8:2 ratio. Further analysis of the NMR data, starting from the proton signal at δ 4.97 br s (H1'), which was indicated to be hemiacetal from the chemical shift of the attached carbon at δ 99.2, allowed us to identify the new 4-amino-4,6-dideoxy-*N*-formyl-2-*O*-3-*C*-dimethyl-β-pyranosyl unit (callipeltose B). A weak COSY correlation between H1' and H2' proved the connectivity between C1' and C2', also confirmed by a ROESY cross peak H1'/H2'. An HMBC cross peak between methoxyl protons signal at δ 3.47 and C2' placed a methoxyl group at C2'. The connectivity C2'/C4' (CH-N, δ 3.97/55.6) *via* the non protonated C3' was established by a combined analysis of HMBC and ROESY spectra. The H2' proton at δ 2.97 showed HMBC correlation to quaternary carbon C3' at δ 68.1, which in turn showed correlations to the methyl singlets at δ 1.44 and 1.42, present in the ratio 8:2. These correlations, in addition to the observed HMBC cross peak between H2' and the methyl carbon at δ 23.8, allowed us to place a tertiary methyl group at C3'. HMBC correlations between the C4' (δ 55.6) and both H2' (δ 2.94 bs) and H8' (δ 1.44) connected C2' to C4' *via* the non protonated C3'. The COSY spectrum showed the H5' (δ 4.11 q, J=6.5 Hz) proton to be coupled to the methyl doublet signal at δ 1.20 (d, J= 6.5 Hz; 1.22, d, J= 6.5 Hz). HMBC correlations from the methyl doublet H6' to C4'(δ 55.6), allowed the C4'-C5' connectivity

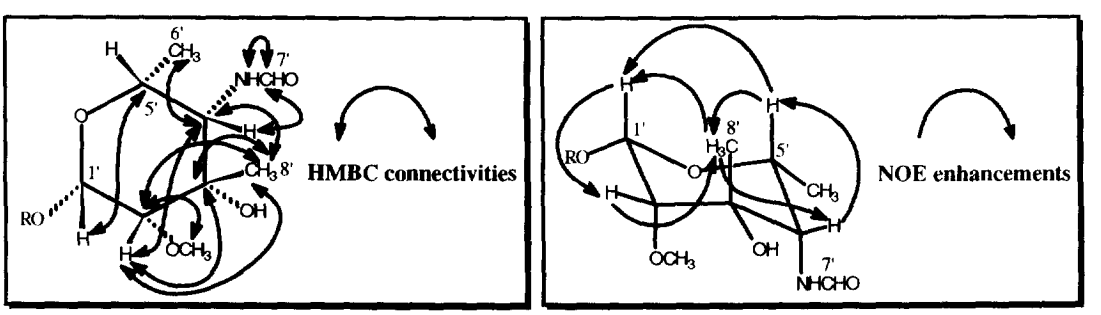

Fig. 1. HMBC and ROESY cross-peaks (mixing time 500 ms) for the sugar portion of callipeltoside B (**2**).

(δ 64.9) to be established. Finally, an HMBC three bond correlation from H1' to C5' closed the pyranose ring. ROESY correlations between H1' and H2', H2' and CH_3-8', H4' to H5' and CH_3-8' revealed their *syn* arrangment. Furthermore, the CH_3-8' methyl signal exhibited ROESY correlations to H1' and H5', indicative of a chair conformation for sugar moiety. This was also confirmed by a ROESY cross peak H1'/H5'. All ROESY data were confirmed by NOE difference experiments (Fig. 1). Therefore, we finally determined the structure of the sugar moiety as 4-ammino-4,6-dideoxy-4-*N*-formyl-2-*O*-3-*C*-dimethyl-β-talopyranosyl, which is glycosidally linked to the C5 of the macrolide on the basis of HMBC correlations from H1' to C5 and from H5 to C1' and a ROESY correlation H1'/H5.

Callipeltoside C [**3**, ESMS *m/z* 675.30-677.30 $(M+Na)^+$, 100, 36] is related to **1** and **2**. Comparison of 1D and 2D-^{1}H NMR and HMQC spectra of callipeltoside C showed identical signals assigned to the macrolide

portion. Differences were observed in the sugar portion. The signals for N-formyl group in **2** were missing in the spectrum of **3**, while were observed signals for a secondary methyl group at δ 1.25 (d, J=6.5 Hz), for a

Table 2. NMR data of callipeltoside C (**3**) in $CDCl_3$ (500 MHz)

Position	δH	δC[a]	Position	δH	δC[a]
1	-	-	20	1.80 m overlapped	34.0
2	2.53 d (12.8), 2.43 d (12.8)	46.0	21	1.29 m	19.0..
3	-	-	22	3.18 m	55.4
4	2.25 dd (11.2, 4.4) 1.36 dt (11.2, 1.30)	43.8	23	1.74 s	15.7
5	3.70 dt (10.2, 4.4)	79.0	24	0.98 d (6.9)	6.1
6	1.50 m	39.8	25	0.93 d (6.4)	12.0
7	3.65 dd (10.5, 2.0)	74.8	OCH_3	3.23 s	55.2
8	2.29 m	38.1	3-OH	4.99 d (1.30)	-
9	3.81 dd (9.4, 2.0)	79.5	1'	4.94 br s	98.9
10	5.30 br dd (9.4)	128.0	2'	3.12 br s	84.7
11	-	-	3'	-	-
12	2.30 dd overlapped 2.09 dd overlapped	46.7	4'	3.36 dd (10.3, 1.20)	76.7
13	5.82 m	71.4	5'	3.65 dq overlapped	66.7
14	5.77 dd (14.8, 6.6)	132.5	6'	1.25 d (6.5)	17.5
15	6.27 dd (14.8, 10.6)	130.6	7'	1.31 s	17.4
16	6.48 dd (15.0, 10.6)	140.0	OCH_3	3.47 s	62.0
17	5.58 dd (15.0, 1.7)	112.1	3'-OH	3.52 d (1.20)	-
18	-	-	4'-OH	2.88 s	-
19	-	-			

[a] Carbon signals detected in HMQC spectrum; small sample prevented the measurement of quaternary carbons by ^{13}C detection.

tertiary methyl group at δ 1.31 and for a methoxyl group at δ 3.47. The major difference in the ^1H NMR dealt with the H4' signal, now observed at δ 3.36 as a doublet with J=10.3 Hz, consistent with an axial proton at C4'. The downfield shift of the associated carbon to 76.7 ppm (*cfr.* 55.6 in **2** and 62.7 in **1**) supported the presence of an equatorial hydroxyl group at C4'. The deuterium exchangeable proton at δ 3.52, (d, J=1.2 Hz), was assigned to OH-4' on the basis of a cross peak between OH-4'/H4' in the COSY. Thus, we propose the structure of 6-deoxy-2-*O*-3-*C*-dimethyl-β-mannopyranose (=2-*O*-methylevalose) for the sugar moiety. D-evalose was found as a constituent of everninomicin B, an oligosaccharide antibiotic produced by *Micromonospora carbonaceae*.[6] The stereochemistry at C2' (axial OCH_3) follows from the coupling constant of the anomeric proton (δ 4.94 br s) and the stereochemistry of anomeric linkage was deduced as β-equatorial from the chemical shift of the acetal carbon at δ 98.9 (cfr. δ 99.2 in **2**). The stereochemistry at C4' and C5' follows from the coupling constant of H4' (J=10.3 Hz), indicative for a vicinal diaxial coupling. Further confirmation of the stereochemistry by NOE experiments was prevented by the small amount of sample available.

Similar to callipeltoside A (**1**), callipeltosides B and C are moderately cytotoxic against NSCLC-N6 (human bronchopulmonary non-small-cell-lung carcinoma) cells with IC_{50} values of 15.1 μg/ml and 30.0 μg/ml, respectively.

Callipeltosides are the first members of a new class of marine-derived glycoside macrolides. The callipeltoside skeleton contains a 14-membered macrocycle lactone linked to a dienyne cyclopropane side chain. The macrocycle includes an hydroxylated hemiketal oxane ring linked to different branched-chain deoxy sugars.

Branched-chain sugars have been reported as constituents of antibiotic macrolides and this may suggest the microbial origin of these compounds. We note that recently a group of glycoside macrolides, polycavernosides, have been reported from a marine source, the toxic red alga *Polycavernosa tsudai*.[7]

Experimental Section

General Information. For general information see: Zampella A. et. al.[3]

Isolation. The sponge *Callipelta* sp. (Demospongiae, Lithistida, Corallistidae) was collected in 1992 and '93 in the shallow waters of 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 Noumea. Preliminary assays for cytotoxic and antifungal (*Fusarium oxysporum, Helminthosporium sativum* and *Phytophtora hevea*) activity showed marked activities for polar and chloroformic extracts (P388 cells, *ca.* 70% inhibition with 10 μg/ml dose).

The organism were freeze-dried and the lyophilized matherial (2.5 Kg) was extracted with *n*-hexane and CH_2Cl_2 in a Soxhlet apparatus, then with CH_2Cl_2 :MeOH 8:2 (3x2 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 by MPLC on a silica gel column (100 g) using a solvent gradient from $CHCl_3$ to $CHCl_3$:MeOH 98:2. The fraction (100 mg) eluted with 99:1 $CHCl_3$/MeOH was further purified by HPLC on a μ-Bondapak-C18 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), 1.0 mg of callipeltoside B (**2**) and 0.8 mg of callipeltoside C (**3**).

Callipeltoside B (**2**): t_r=11.2 min, ^1H and ^{13}C NMR in the Table 1; ESMS *m/z* 702.36-704.34 [$(M+Na)^+$, 100, 36]. UV (MeOH): λ 250 (ε 6000), 272 (ε 8132), 286 nm (ε 6340);

Callipeltoside C (**3**): t_r=7.2 min; ^1H and ^{13}C NMR in the Table 2; ESMS *m/z* 675.30-677.30 [$(M+Na)^+$, 100, 36].

Determination of biological activity.

Cytotoxic assays. Experiments are performed in 96 wells microtiter plates ($2x10^5$ cells/ml). Cell growth is estimated by a colorimetric assay based on conversion of tetrazolium dye (MTT) to a blue formazan product using live mitochondria.[8] Eight determinations are performed for each concentration. Control growth is estimated for 16 determinations. Optical density at 570 nm corresponding to solubilized formazan is read for each well on Titertek Multiskan MKII.

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