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Isolation of callipeltins A–C and of two new open-chain derivatives of callipeltin A from the marine sponge *Latrunculia* sp. A revision of the stereostructure of callipeltins

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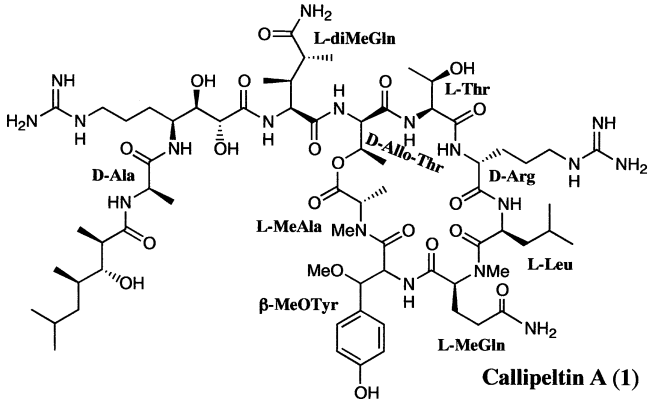
Abstract—Two new callipeltin-related acyclic peptides (**2** and **3**) have been isolated, together with callipeltins A–C from the marine sponge *Latrunculia* sp. collected at the Vanuatu Islands. The gross structures of new compounds were elucidated by spectroscopic data. The application of the Marfey's analysis on the new derivatives and on callipeltin A (**1**), allowed us to revise the configuration of two amino acid units in callipeltin A.

The novel cyclodepsipeptide callipeltin A[1] (**1**) and its congeners[2] were isolated in our laboratories from the marine sponge *Callipelta* sp., collected in New Caledonia. Callipeltin A is a decapeptide, containing three unusual amino acid residues: β-methoxytyrosine (βOMeTyr), (2*R*,3*R*,4*S*)-4-amino-7-guanidino-2,3-dihydroxyheptanoic acid (AGDHE), and (3*S*,4*R*)-3,4-dimethyl-L-glutamine (diMeGln). Callipeltin A (**1**) is known to exhibit antifungal and anti-HIV activity. Recently it was found that callipeltin A is a selective and powerful inhibitor of the Na/Ca exchanger and a positive inotropic agent in guinea pig left atria.[3]



Callipeltin A (1)

Continuing our investigation on the marine sponges collected in the Vanuatu Islands, we had the opportunity to study the sponge *Latrunculia* sp. whose ethanolic extracts showed a marked cytotoxicity against Kb cells (100% inhibition at 10 μg/mL). The major components of the polar extracts of the sponge were found to be callipeltins A (**1**)–C. In addition, two new truncated open-chain derivatives of callipeltin A, named callipeltins D and E (**2** and **3**) were isolated.

In this paper, we describe the isolation and the structure determination of the new compounds along with the revision of the stereostructures of the callipeltins A (**1**)–C.

The lyophilized sponge was extracted with methanol and the crude methanolic extract was subjected to a modified Kupchan's partitioning procedure. Fractionation of the chloroformic-soluble material (1.45 g) by DCCC ($CHCl_3$/MeOH/H_2O 7:13:8 ascending mode) followed by reversed phase HPLC (Vidak, CH_3CN/H_2O, 30–32% 0.1% TFA) afforded callipeltins C, A and B (0.01, 0.1, 0.005% w/w, respectively) in this order of elution from DCCC. Fractionation of the butanolic-soluble material (1.6 g) by DCCC and reversed phase HPLC in the same conditions used for the chloroformic extract furnished callipeltins D, **2** (6.7 mg) and E, **3** (4.3 mg).

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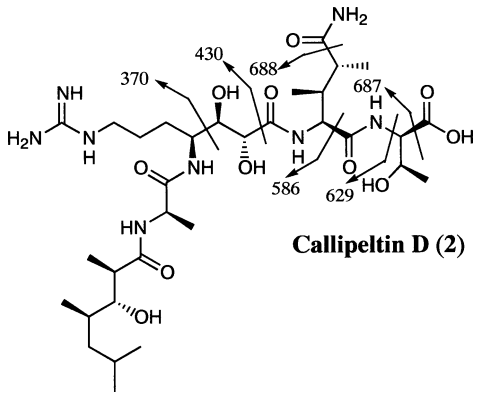
Table 1. ^{1}H and ^{13}C NMR data (500 MHz, CD_3OD) for compounds **2** and **3**.

Callipeltin D (**2**)			Callipeltin E (**3**)		
aa	δ_H[a]	δ_C	aa	δ_H	δ_C
	Thr			MeAla	
α	4.46 br s	60.0	α	5.15 q (7.7)	54.2
β	4.16 overlapped	68.8	β	1.41 d (7.7)	14.7
γ	1.30 d (6.0)	20.1	NMe	2.86 s	32.6
CONH		172.3	CO		172.1
	DiMeGln			βMeOTyr	
α	4.56 d (7.3)	57.6	α	5.21 d (10.1)	54.6
β	2.18 m	39.5	β	4.33 d (10.1)	87.5
βMe	1.04 d (6.8)	14.0	C4		129.2
γ	2.63 m	42.2	C5/C9	7.19 d (8.7)	130.4
γMe	1.20 d (7.0)	19.9	C6/C8	6.80 d (8.7)	116.1
CONH		173.1	C7		117.4
$CONH_2$		180.3	OMe	3.15 s	57.0
	AGDHE		CONH		171.4
α	4.02 d (7.1)	73.4		MeGlu	
β	3.76 dd (7.1, 2.7)	75.3	α	4.87 m	57.5
γ	4.16 overlapped	51.0	β	1.98, 1.72 m	26.3
δ	1.67, 1.30 m	26.4	γ	1.99 m	32.6
ε	1.67 m	26.5	NMe	3.01 s	30.8
ζ	3.25 m	42.0	CO		173.4
CO		175.4	$CONH_2$		180.1
Guan		155.6		Leu	
	Ala		α	4.78 dd (10.5, 4.4)	50.0
α	4.33 q (7.3)	51.1	β	1.63 m	40.9
β	1.42 d (7.3)	17.8	γ	1.63 m	25.9
CONH		176.8	Me-γ	0.99 d (6.6)	23.6
	TMHEA		Me-ψ	0.98 d (6.6)	21.6
1		177.2	CONH		175.0
2	2.63 m	44.9		Arg	
3	3.52 dd (8.7, 2.7)	79.9	α	4.42 t (7.8)	54.2
4	1.77 m	34.0	β	1.97, 1.65 m	25.9
5	1.26 m	40.4	γ	1.60 m	26.3
6	1.69 m	24.8	δ	3.20 m	41.9
7	0.98 d (6.2)	24.8	CONH		176.3
8	1.10 d (6.0)	14.4	Guan		158.9
9	1.01 d (6.5)	17.5		Thr	
10	0.90 d (6.5)	21.8	α	3.90 d (4.3)	59.2
			β	4.20 m	67.1
			γ	1.29 d (7.0)	18.7
			CONH		172.0

[a] Coupling constants are in parentheses and given in Hz. ^{1}H and ^{13}C assignments aided by COSY, TOCSY, HMQC and HMBC experiments.

Callipeltin D **2** {$[\alpha]_D^{25}$ −9 (*c* 0.14, MeOH)}was obtained as colorless amorphous solid and showed the pseudo-molecular ion peak at *m/z* 733.4432 $(M+H)^+$ in the HRFABMS spectrum, corresponding to the molecular formula $C_{32}H_{60}N_8O_{11}$ (Δ +2.8 mmu). Extensive analysis of the ^{1}H and ^{13}C NMR data of **2**, including ^{1}H–^{1}H COSY, HMQC, HMBC spectra (see Table 1) by comparison with those of callipetins A–C, disclosed the presence of one residue each of alanine (Ala), threonine (Thr), 3,4-dimethylglutamine (diMeGln), 4-amino-7-guanidino-2,3-dihydroxyheptanoic acid (AGDHE) and 3-hydroxy-2,4,6-trimethylheptanoic acid, also present in callipeltin A (**1**). The sequencing of these units was carried out by a combination of HMBC and FABMS data. The attachment of the 3-hydroxy-2,4,6-trimethylheptanoyl residue to the α-amino group of the Ala residue was indicated by a HMBC cross peak between the Ala α-proton and the acyl group of the heptanoyl residue. The remaining sequence, which was found to be the same present in the corresponding subunit in callipeltin A (**1**), was determined by interpretation of the fragmentation peaks observed in the FABMS spectrum, reported in Fig. 1.

Absolute stereochemistry of the conventional amino acid residues was determined by HPLC analysis of the acid hydrolyzate derivatized with Marfey's reagent.[4] By using the buffer-free elution conditions recently developed in our laboratories,[5] we obtained a well resolved HPLC trace. Surprisingly, no peaks ascribable to L-Thr and to L-Ala were observed, whereas one residue of D-Allo-Thr and D-Ala was detected. The *S* configuration of the C-4 stereocenter in the AGDHE residue was confirmed to be the same as in callipeltin A by using the same procedure (oxidative cleavage followed by acid hydrolysis) used for callipeltin A.[1]



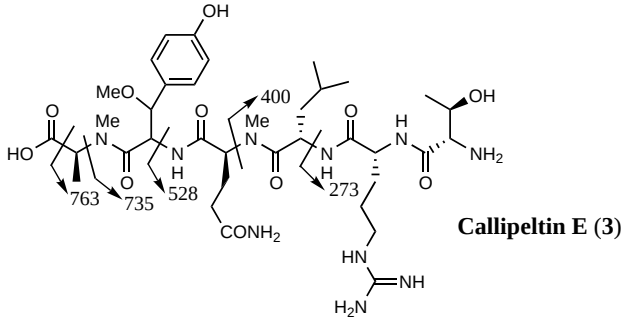
Callipeltin D (2)

Figure 1. Callipeltin D (**2**) with FAB MS fragmentations.

The molecular formula, $C_{36}H_{61}N_{10}O_{11}$, of callipeltin E (**3**) $\{[\alpha]_D^{25}$ −46 (*c* 0.1, MeOH)} was determined by HR-FABMS [*m*/*z* 809.4537 $(M+H)^+$, Δ +1.6 mmu]. Analysis of 1D and 2D NMR (COSY, HOHAHA, HMQC, HMBC) data delineated six spin systems which could be ascribed to six amino acid residues [Thr, Arg, Leu, MeGln, β-MeOTyr, MeAla] which were also present in callipeltin A. The sequencing of these units by the analysis of FAB-MS fragmentation peaks led to the structure as shown in Fig. 2.

The stereochemistry of the amino acid residues was determined by Marfey method to be L-Thr, D-Arg, L-Leu, L-MeGln, L-MeAla. The configuration of the β-methoxytyrosine residue remains unassigned because this fragment was lost during acid hydrolysis.

The finding of D-AlloThr and D-Ala in callipeltin D (**2**) prompted us to re-examine the stereochemistry of the conventional amino acid residues in callipeltin A (**1**). Therefore, callipeltin A (**1**) was subjected to acid hydrolysis and the hydrolyzated was derivatized with Marfey's reagent followed by HPLC analysis. The HPLC trace showed peaks for L-Thr (12.7 min), D-Arg (14.4 min), L-MeAla (14.4 min), L-MeGlu (14.8 min), D-Ala (19.2 min) and L-Leu (26.3 min). As previously reported,[5] Ala and NMeAla undergo a significative racemization during acid hydrolysis, as evidenced by the presence of two additional peaks at 16.4 min (L-Ala) and 18.3 min (D-NMeAla), respectively, in the HPLC trace. Because the D-AlloThr showed the same retention time (14.4 min) of D-Arg and L-NMeAla in the HPLC elution conditions used in the Marfey analysis, at this point we were unable to define the presence in callipeltin A (**1**) of two L-Thr residues or, alternatively, of one residue each of L-Thr and D-AlloThr. To solve this ambiguity, we performed the Marfey's analysis on two fractions, obtained from the DCCC fractionation of the acid hydrolysate of callipeltin A (**1**),[6] both containing Thr as evidenced by ^1H NMR analysis. The Marfey's analysis revealed that the less polar fraction from DCCC contained D-Ala and D-AlloThr, whereas the other fraction was composed by L-Thr.



Callipeltin E (3)

Figure 2. Callipeltin E (**3**) with FAB MS fragmentations.

Therefore the stereostructure of callipeltin A (**1**), that we have originally proposed, should be revised by replacing the L-Ala residue with D-Ala and one of two Thr residues with D-AlloThr.

Comparison of the chemical structures of callipeltin A (**1**) with those of callipeltin D (**2**) and E (**3**) makes well founded the hypothesis that D-AlloThr residue in callipeltin D and the L-Thr residue in callipeltin E correspond to the acylated and non acylated Thr residues in callipeltin A, respectively, leading to the revised structure **1**.

In order to gain insights into the structural relationships responsible for the potent inotropic activity and for the inhibition of the cardiac Na/Ca exchanger, previously observed for callipeltin A (**1**), we have investigated the effect of callipeltins B–D in cardiac preparations.[7] Whereas in cardiac sarcolemmal vesicles callipeltins C and D (**2**) (0.5, 1.4 and 10 μM) did not show significative inhibitory activity on the Na/Ca exchanger, callipeltin B displayed a weak inhibition activity (−23% inhibition at 4 μM). At the same concentrations, callipeltins B–D did not induce positive inotropic effect, even if a significant rise in resting tension was observed when callipeltin C was added to the incubation medium at concentration higher than 3 μM. The increase of resting tension, also observed in callipeltin A (**1**), is an expression of Ca^{2+} overload in the cardiac myocytes. Further studies are needed to explain why callipeltin C causes this effect without inducing a positive inotropic effect.

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6. Experimental details on the fractionation of the acid hydrolysate were reported in Ref. 1.
7. For the methods used see Ref. 3.