55

10657

Pergamon

Tetrahedron Letters, Vol. 36, No. 48, pp. 8853-8856, 1995
Elsevier Science Ltd
Printed in Great Britain
0040-4039/95 $9.50+0.00

0040-4039(95)01837-9

Caledonin, a Natural Peptide Bolaphile with Zn^{II} and Cu^{I} Complexing Properties from the Tunicate *Didemnun rodriguesi*

María Jesús Vázquez[1], Emilio Quiñoá[1] and Ricardo Riguera[1]*
Anuncia Ocampo[2] and Teresa Iglesias[2]
Cécile Debitus[3]

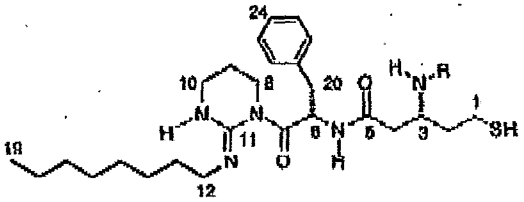
[1]Departamento de Química Orgánica, Facultad de Química, Universidad de Santiago, 15706 Santiago de Compostela, Spain, Tel/FAX 34-81-591091

[2]Facultad de Ciencias, Universidad de Vigo, Vigo, Spain

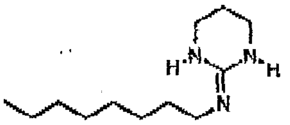
[3]Centre ORSTOM, B. P. A5, Nouméa Cedex, New Caledonia

Abstract: Caledonin (1) is a modified peptide isolated from the marine tunicate *Didemnun rodriguesi*. Caledonin comprises a central L-phenylalanine residue connected via its amino group to *(S)*-3-amino-5-mercaptopentanoic acid (a new sulfur-containing β-amino acid), and by its carboxyl group to a six membered cycloguanidine moiety, bearing an *n*-octyl chain. Caledonin is a natural bolaphile which strongly binds Zn^{II} and Cu^{I} ions.

In the last decades, tunicates have been subjected to very close scrutiny and as a result, a large number of bioactive nitrogenous metabolites with significant biological activity have been described.[1] They include acyclic and cyclic peptides and depsipeptides with novel and complex structures and also compounds of physiological importance characterized by the presence of cyclic guanidine groups.[2,3] As part of our work on pharmacologically active metabolites from marine organisms, we now report the structure of caledonin (1), a novel modified peptide, which was isolated from the tunicate *Didemnun rodriguesi*. Caledonin, among other relevant structural features, possesses a new β-amino acid residue, a cycloguanidine group and presents strong metal-complexing properties.



1 R = H
3 R = (R)-COCH(OMe)Ph
4 R = (S)-COCH(OMe)Ph

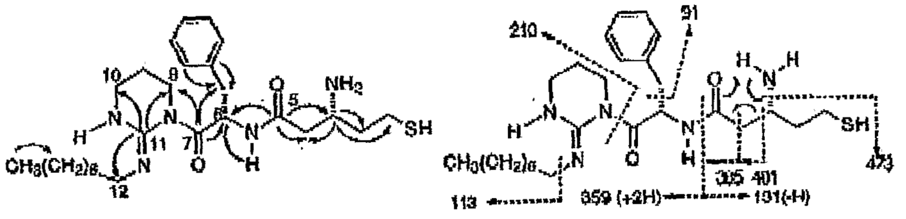


2

Didemnun rodriguesi (Tunicata, Didemnidae),[4] an encrusting ascidian of a deep pink, red or yellow colour, was collected from the Baie des Citrons, Nouméa (New Caledonia). We isolated[5] pure caledonin (1) [48 mg; mp= 170-172°C; $[\alpha]_D^{20}$ = +24 (MeOH, c=1.45 mg/mL)] from the methylene chloride soluble material that showed *in vitro* cytotoxicity against Kb cells (85% inhibition at 10 μg/mL). The IR spectrum[6] showed characteristic absorption bands due to amide groups [(KBr) 1656, 1630 cm^{-1}] and the UV indicated the presence of a phenyl chromophore [(MeOH) λ_{max} 234 (ε 2816), 280 (ε 666; sh) nm]. Its (+) FABMS (glycerol) spectrum showed a peak corresponding to the molecular ion at m/z 489 ($[M]^+$), confirmed when NaCl was added to the matrix (m/z 511, [Na(M-H)]$^+$). Its EIMS showed the [M-H]$^+$ ion at m/z 488 and so did the HREIMS (m/z 488.30650, Δ= +1.2 ppm, corresponding to $C_{26}H_{42}N_5O_2S$, 8 unsaturations).

One- (^{1}H, ^{13}C, DEPT) and two-dimensional (DQF-COSY, TOCSY, HMQC) NMR analysis revealed the presence of four main substructures generating independent ^{1}H spin systems, and linked by one C-N and two peptide bonds. Those fragments were: 1) a new β-amino acid (3-amino-5-mercaptopentanoic acid; 2) phenylalanine; 3) a guanidine group with two nitrogens linked by a $-CH_2CH_2CH_2-$ chain and forming a six membered ring and 4) an *n*-octyl chain. These substructures satisfy the unsaturation requirements (6 double bonds and 2 rings) suggested by the molecular formula.

Diagnostic ^{13}C and ^{1}H shifts (Table 1) showed the nature of the different heteroatoms and functional groups present in the molecule: one primary amine bound to a methine (δ 61.8, δ 3.24, 3-CH) with γ heteroatom substituents, one guanidine group (δ 160.2, 11-C), two amide groups (δ 171.3, 5-C; δ 166.0, 7-C) and finally, the characteristic shifts of 1-CH_2 (δ 26.9, δ 1.10) indicate a thiol group attached to that position. Exchangeable ^{1}H NMR spectra ($CDCl_3/D_2O$) confirmed that the broad singlets at δ 4.8 (4H) and δ 6.0 (1H) were originated by hydrogens attached to heteroatoms; and ^{1}H-^{15}N HMQC and COSY experiments identified the signal at δ 6.0 as the NH attached to the 10-CH_2.



a b

Figure 1. a. Selected HMBC ($^2J_{CH}$ and $^3J_{CH}$) correlations. b. Selected (+) FABMS fragmentations.

The precise connectivities between the substructures were established by HMBC and corroborated by ROESY experiments. Selected correlations are shown in Figure 1a. Among them, those between 5-CO and 6-CH; 7-CO and 8-CH_2; and 11-C and the CH_2 groups at positions 8, 10 and 12, were the most useful. Caledonin was thus shown to comprise a central phenylalanine residue flanked by a 3-amino-5-mercaptopentanoic acid and the cycloguanidine moiety, with the *n*-octyl chain bound to the exo-nitrogen. Furthermore, the observed (+) FABMS fragmentation patterns are in good agreement with that structure (Figure 1b).

Several chemical transformations were carried out to confirm the structure of caledonin. Acid hydrolysis of 1 (6N HCl; 12h at 110°C) yielded three products identified (^{1}H-NMR, MS) as phenylalanine, 3-amino-5-mercaptopentanoic acid and the substituted guanidine 2. Derivatization (1. *n*-BuOH; 110°C, 2. TFAA; 150°C) followed by chiral GC-MS analysis (Chirasil Val III; He at 1 mL/min; 120 to 200°C) indicated that the phenylalanine belonged to the L-series (*S* absolute configuration). Derivatization of caledonin with (*R*)- and (*S*)-methoxyphenylacetic acid (MPA), afforded after HPLC separation, the MPA amides 3 and 4. Analysis of their ^{1}H-NMR spectra[7] indicated the absolute configuration at 3-C to be *S* (Figure 2a).[8]

Tunicates are known to produce metal complexing metabolites, such as the tunichroms.[9] Some features of caledonin, and in particular the hydrophobic chain at one end and the penicillamine-like β-amino acid[10] at the other, suggest that this metabolite is a natural bolaphile[11] which may be involved in ion transport across membranes.

To test this hypothesis, a solution of caledonin in CD_3OD was titrated with $ZnCl_2$ and monitored by ^1H-NMR spectroscopy. The 2:1 caledonin/$ZnCl_2$ adduct was isolated and characterized by (+) FABMS (m/z 552, $[Zn(M-H)]^+$) and NMR spectroscopy (^1H, ^{13}C, COSY, TOCSY and HMQC experiments).

Table 1. ^1H and ^{13}C NMR data for caledonin (1) [a]

At. no.	^{13}C (δ)	^1H (δ),*m*, J(Hz)	At. no.	^{13}C (δ)	^1H (δ),*m*, J(Hz)
1	26.9	1.10, dd (7.1, 13.1)	12	40.6	2.68, m
2	31.7	1.55 1.42	13	31.0	1.75, dd (7.5, 15.1)
3	61.8	3.24, m	14-17	29.3-29.6	1.38-1.55
4	39.0	3.16, t (12.1) 2.12, dd (8.1, 14.1)	18	22.7	1.38
5	171.3		19	14.1	0.89, t (6.6)
6	66.3	4.50, t (1.0)	20	35.6	3.39, dd (4.4, 13.6) 3.20, dd (2.0, 13.5)
7	166.0		21	134.8	
8	52.4	3.53, d (17.0) 2.79, m	22/26	130.5	7.16-7.26
9	27.4	1.55	23/25	128.6	7.16-7.26
10	38.9	3.75, m 2.75, d (17.0)	24	127.7	7.16-7.26
			11-NH		6.00, s
11	160.2		NH(4H)		4.80, bs

[a] NMR specra were recorded on a Bruker AMX-500 spectrometer in $CDCl_3$ at 298 K.

Comparison of the ^1H-NMR data of 1 and its Zn^{II} complex (Table 2) showed that the thiol and amino groups are involved in complex formation with the Zn^{II} ion in a tetrahedal environment, as shown in Figure 2b.

Very similar ^1H-NMR spectrum was obtained when 1 was titrated with CuCl, suggesting formation of a similarly bonded adduct, but the chelated Cu^I ion was rapidly oxidized to Cu^{II}, hampering isolation and precise structure determination.

Table 2. Selected NMR data for caledonin (1) and for caledonin-Zn^{II} complex in CD_3OD.

	1		1-Zn^{II} complex	
At. no.	^1H (δ)	^{13}C (δ)	^1H (δ)	^{13}C (δ)
1	1.10	27.55	1.18	29.72
2	1.61/1.52	35.75	1.65/1.33	35.51
3	3.24	49.00	4.37	51.85
4	3.16/2.12	40.48	2.41/2.22	41.88

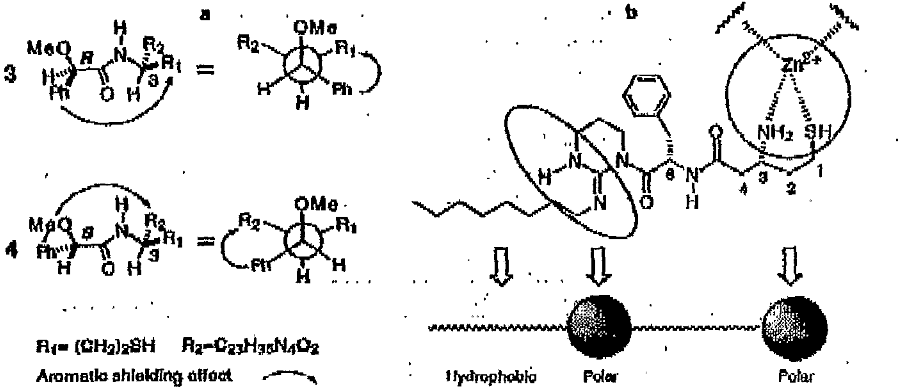


Figure 2. a. Perspective and elongated Newman projections for **3** and **4** showing the magnetic shielding effect[8] based on Ref. 7. b. Caledonin-Zn^{II} complex, showing its bolaphile characteristics.

Acknowledgements

This work was financially supported by the CICYT (SAF 92-1023) and the Xunta de Galicia (XUGA 20907B/93 and XUGA 20907B/94).

REFERENCES AND NOTES

1. Ireland, C. M.; Molinski, T. F.; Roll, D. M.; Zabriskie, T. M.; Mckee, T. C.; Swersey, J. C.; Foster, M. P.; in *Bioorganic Marine Chemistry*, vol 3, Scheuer, P.J. Ed., Springer-Verlag, Berlin Heidelberg, **1989**, pp. 1-46.
2. Ohtani, I.; Kusumi, T.; Kakisawa, H.; Kashman, Y.; and Hirsh, S. *J. Am. Chem. Soc.*, **1992**, *114*, 8472.
3. Ohtani, I.; and Moore, R.; *J. Am. Chem. Soc.*, **1992**, *114*, 7941.
4. Da Rocha, R.M.; and Monniot, F. *Ann. Inst. Océanogr., Paris*, **1993**, *69*, 261-265.
5. Freshly collected tunicate was frozen on site, transferred over dry ice and then lyophilized. 1 Kg of lyophilizate was homogenized in MeOH and extracted at r.t. for four days (4 x 2 L). The MeOH was evaporated *in vacuo* and the residue was partitioned between water and CH_2Cl_2. 5 g of the CH_2Cl_2-soluble material were successively chromatographed on silicagel (CH_2Cl_2-MeOH) and Sephadex LH-20 (MeOH) columns, and then on reversed-phase HPLC (μ-Bondapack C_{18} column; MeOH:H_2O 95:5; flow: 4 mL/min; retention time 12 min; RI detector), to afford 48 mg of pure caledonin (**1**).
6. IR (KBr) 2922, 2852, 1656, 1630, 1548, 1457 cm^{-1}.
7. Latypov, S. K.; Seco, J. M.; Quiñoá, E.; and Riguera, R. *J. Org. Chem.*, **1995**, *60*, 1538.
8. For instance, characteristic shifts for the pair of derivatives **3**(**4**) were δ 4.93(4.65) and 2.21(2.18) for 6-H and 4-H respectively.
9. a) Smith, M. J.; Kim, D.; Horenstein, B.; and Nakanishi, K. *Acc. Chem. Res.*, **1991**, *24*, 117.
   b) Michael, J. P.; and Pattenden, G. *Angew. Chem. Int. Ed. Engl.*, **1993**, *32*, 1.
10. Penicillamine is a well known antirheumatic and chelating agent for copper in Wilson's disease: Merck Index, 11th Edition, Merck&Co., Inc. Rahway, N.J., U.S.A., 1989, p. 7029 and references therein. Caledonin also resembles the antihypertensive captopril, Merck Index, 11th Edition, Merck&Co., Inc. Rahway, N.J., U.S.A., 1989, p. 1773 and references therein.
11. a) Fuhrhop, J.-H.; and Fritsch, D. *Acc. Chem. Res.*, **1986**, *19*, 130, and references therein.
   b) Escamilla, G. H.; and Newkome, G. R. *Angew. Chem. Int. Ed. Engl.*, **1994**, *33*, 1937, and references therein.

(*Received in UK* 13 *July* 1995; *revised* 27 *September* 1995; *accepted* 29 *September* 1995)