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Onchidin: A Cytotoxic Depsipeptide with C_2 symmetry from a Marine Mollusc.

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Abstract: Onchidin (1) is a cytotoxic depsipeptide isolated from the pulmonate mollusc *Onchidium* sp. Its structure is cyclo (MeVal-Amo-Val-Hiv-Hiv-MeVal-Amo-Val-Hiv-Hiv) made of two identical halves ($C_{30}H_{49}N_3O_7$, MeVal-Amo-Val-Hiv-Hiv). Onchidin contains a new β-amino acid: 3-amino-2-methyl-oct-7-ynoic acid (Amo). It has C_2-symmetry and this makes one half homotopic to the other so only signals for a "monomer" can be seen in the NMR spectra. The structure and absolute stereochemistry (all S) of onchidin was determined by extensive spectroscopic analysis, selective hydrolysis and chiral GCMS.

Cyclic peptides with important pharmacological properties have been isolated from marine organisms.[1] Frequently these metabolites contain D amino acids, hydroxy acids, new α-amino acids, new β-amino acids and thiazole and oxazole rings. In some cases, the cyclic structure incorporates elements of symmetry that complicate the structural determination due to the bonding possibilities of the repeating fragments and the simplification of the spectra. The interest of these compounds is well illustrated by dolastatin-10, the most potent antineoplastic agent known[2] and by the didemnins,[3] considered as some of the most promising antitumor compounds of marine origin. In this paper we describe the structure (including absolute configuration) of onchidin (**1**) a new cytoxic depsipeptide with C_2 symmetry isolated from a pulmonate mollusc known to be a rich source of γ-pyrone polypropionates[4].

Onchidium sp. (*Pulmonata*, order: *Stylommatophora*) (3Kg. fresh weight) collected off New Caledonia was extracted with methanol. P-388 and Kb activity-guided fractioning by solvent partition followed by repeated chromatography (Sephadex, reverse phase HPLC) afforded 3 mg of a pure compound ($[\alpha]_{25}^{D}$=-140.9°; P-388 IC_{50}=8 μg/mL) that we named onchidin (**1**).

The ^{13}C/DEPT NMR showed the presence of 30 signals. They are 5 amide or ester carbonyls (δ=172.2, 171.7, 170.6, 169.6, 169.5 ppm), 9 Me signals (from δ=22.0 to 14.7 ppm), one NMe (δ=40.4 ppm), 3 CH_2 (δ=33.2, 24.7, 17.8 ppm), 6 CH bonded to oxygen or nitrogen (from δ=79.7 to 44.7 ppm), 4 isopropyl-type CHs (from δ=30.8 to 28.3 ppm) and other two carbons (δ=84.4 and 68.4 ppm) that could be assigned to a terminal acetylene.[5]

The ^{1}H-NMR (500 MHz, $CDCl_3$) showed two NH doublets (δ=7.67 and 6.04 ppm), 6 signals corresponding to the α-amino and α-hydroxy acid methine protons (from δ=5.24 to 2.67 ppm), one NMe (δ=3.39 ppm), 3 CH_2 (from δ=2.24 to 1.27 ppm), 4 isopropyls groups (8 Me from δ=1.20 to 0.98 ppm), 4

CH from δ=2.65 to 2.14 ppm) and one additional secondary Me (δ=1.25 ppm, doublet). The terminal acetylene CH was detected at δ=1.91 ppm (J=2.0 Hz) as a triplet due to propargylic coupling.

HMQC, COSY and TOCSY established the presence of the following five independent spin systems: two 2-hydroxy isovaleric acids (Hiv), one valine, one N-methylvaline and a new β-amino acid: 3-amino-2-methyl-oct-7-ynoic acid (Amo), which comprises two tertiary chiral centers substituted with a methyl and a pentine side chain (Table 1).

Table 1. NMR data for onchidin ($CDCl_3$, 500 MHz for ^1H and 125 MHz for ^{13}C)

C#	^1H, m (J in Hz) [a]	^{13}C [b]	C#	^1H, m (J in Hz) [a]	^{13}C [b]
MeVal			**Val**		
1		171.7[c]	15		172.2
2	3.29, d (7.99)	72.3	16	4.38, dd (9.5; 6.0)	60.1
3	2.65, dh (8.0; 7.0)	28.3	17	2.41, dh (6.0; 7.0)	30.0
4	1.01, d (8.0)	14.7[d]	18	1.02, d (7.5)	16.9[d]
5	1.20, d (6.5)	22.7	19	1.02, d (7.0)	18.5[d]
NMe	3.39, s	40.4	NH	7.63, d (9.5)	
Amo			**Hiv 1**		
6		170.6[c]	20		169.5
7	2.67, dq (2.0; 7.0)	44.7	21	5.12, d (2.5)	79.7
8	1.25, d (7.0)	16.3[d]	22	2.51, dh (2.5; 7.0)	29.4
9	4.13, dddd (10.0;10.0;5.0; 2.0)	51.8	23	1.02, d (7.0)	19.1[d]
10	1.75, m	33.2	24	1.00, d (7.0)	19.4[d]
	1.59, m				
11	1.27, m	24.7	**Hiv 2**		
12	2.24, dddd (16.5; 7.0; 7.0; 2.5)	17.8			
	2.14, ddd (19.5; 7.0; 7.0)		25		169.6[c]
13		84.4	26	5.24, d (5.0)	73.4
14	1.91, t (2.0)	68.4	27	2.14, dh (6.0; 6.5)	30.8
NH	6.04, d (9.9)		28	1.05, d (7.0)	19.6[d]
			29	0.98, d (7.0)	19.9[d]

[a] Assignments based on COSY and TOCSY. [b] Assignments based on HMQC and HMBC.
[c, d] Assignments for these signals may be interchanged.

The connectivities among those residues were deduced from ROESY and LRCOSY experiments, (Figure 1) and support the presence of the structural unit made of MeVal-Amo-Val-Hiv-Hiv.

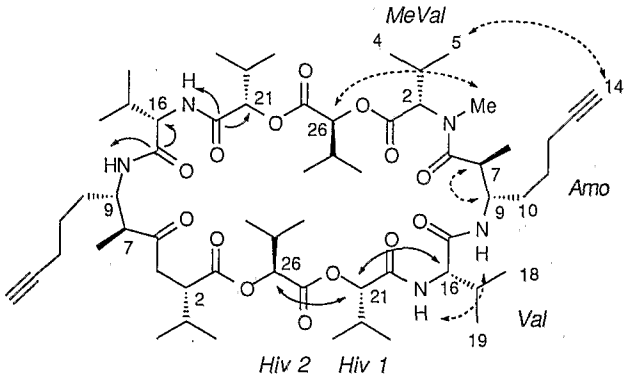

Fig. 1. Selected NOE ( ), LRCOSY ( ) and HMBC ( ) for **1**.

Although the EIMS of **1** shows the highest mass fragment at *m/z* 563.3559 corresponding to $C_{30}H_{49}N_3O_7$, the (+)FABMS showed clearly that the molecular formula of **1** is twice that. Thus (+)FABMS taken in NBA matrix showed an abundant $[M+H]^+$ peak at *m/z* 1127 (15%), corresponding to a dimeric formula $C_{60}H_{99}N_6O_{14}$ (8 unsaturations) and the base peak at *m/z* 564 ($[C_{30}H_{49}N_3O_7+H]^+$, 100 %). It shows also ions at *m/z* 647 and 663. The fragment at *m/z* 647 $[564+C_5H_7O]^+$ originates from a McLafferty type rearrangement of Hiv (Figure 2). The ion at *m/z* 663 $[564+C_5H_9NO]^+$ corresponds to a six unit fragment (monomer bonded to Val). Other relevant peaks in this (+)FABMS are those at *m/z* 214 [Hiv-MeVal+H]$^+$ and 314 [Hiv-Hiv-MeVal+H]$^+$. Tandem FABMS/MS on *m/z* 564 is in perfect agreement with that structure (Figure 3).

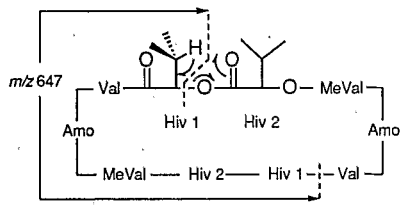

Fig. 2. Mc Lafferty rearrangements *m/z* 647.

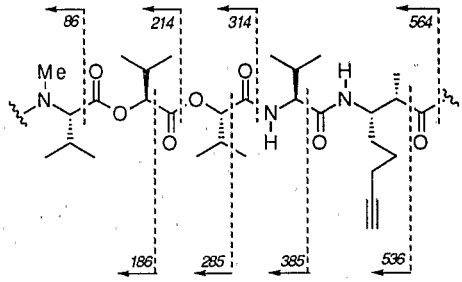

Fig. 3. Tandem FABMS/MS on *m/z* 564.

A (+)FABMS (Figure 4) taken in another matrix (2-hydroxyethyl disulfide + NaCl) corroborated those results showing the molecular ion (*m/z* 1127 $[M+H]^+$ and 1149 $[M+Na]^+$) and an ion at *m/z* 657 $[564+C_6H_7N]^+$ originated by a McLafferty rearrangement (Figure 5) involving one Amo unit.

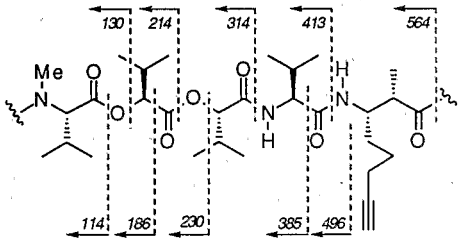

Fig. 4. (+)FABMS of **1**

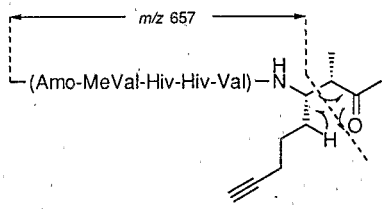

Fig. 5. Mc Lafferty rearrangements *m/z* 657.

Thus, onchidin $C_{60}H_{99}N_6O_{14}$ is a dimer[6] of the $C_{30}H_{49}N_3O_7$ structural unit (MeVal-Amo-Val-Hiv-Hiv) with a high degree of symmetry that provokes the coincidence of NMR signals. As only 7 unsaturations can be explained by the ^{13}C NMR data it must have a ring and its structure is cyclo (MeVal-Amo-Val-Hiv-Hiv-MeVal-Amo-Val-Hiv-Hiv) **1**.

The sequence of amino acids and the exact way of bonding of the two halves of the molecule was determined unambiguously by analysis of the carbonyl group correlations in the HMBC spectrum. Thus the signal at higher field (δ=169.5 ppm, C-20) shows strong correlations with the NHVal and with H-21, and a weak one with H-16. The lowest field C=O signal (δ=172.2 ppm, C-15) correlates strongly with both H-16 and NHAmo. Finally, basic hydrolysis of **1** afforded Hiv and the expected tetrapeptide identified by EIMS.

Acid hydrolysis of **1** followed by chiral GC-MS analysis proved that all the α-hydroxy and amino acids, belong to the L series (S absolute stereochemistry). The new β-amino acid (Amo), was found to be threo on the

basis of the NOE observed between H-7 and H-9 and their coupling constant (2.0 Hz). The NOEs between H-10 and the Me-4, Me-18 and Me-19, and between H-14 and Me-5 are in coincidence with the threo disposition and indicate that the pentine side chain lies on the same side as the MeVal and Val isopropyl groups. As a result, the absolute configuration is (7S, 9S) and onchidin is the all S depsipeptide: cyclo (MeVal-Amo-Val-Hiv-Hiv-MeVal-Amo-Val-Hiv-Hiv), a dimeric structure made of two identical halves ($C_{30}H_{49}N_3O_7$, MeVal-Amo-Val-Hiv-Hiv). This molecule has C_2-symmetry and this makes one half homotopic to the other so that only signals for a "monomer" can be seen in the NMR spectra.

Only very few cyclic peptides and depsipeptides from natural origin incorporate elements of symmetry. Barettin (12-membered ring, dimeric)[7] and cycloxazoline (westiellamide) (18-membered ring, trimeric)[8] from sponges are the only examples reported in the marine metabolite field. Onchidin (**1**), constitutes the first report of a dimeric depsipeptide from a mollusc. It is also the largest symmetric peptide isolated from any marine source.

In contrast to the fair number and variety of cyclic peptides reported from microscopic algae, sponges and ascidians, the dolastatins[9] are to this date the sole precedent in molluscs. Onchidin recalls the structure of valinomycin, from *Streptomyces fulvissimus* (36-membered ring, trimeric, made of L-Val, D-Hiv, D-Val and L-Lac) that is a well known antibiotic and antiparasitic compound with K^+ complexing properties[10].

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