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Identification and ET Comparison of Venom Toxin Proteins of Puff Adder and African Bush Viper by Mass Spectrometrum

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Abstract

Tropical and equatorial Africa is a paradise for the herpetologist. Many species of reptiles are present, but often difficult to discover and observe [1]. With an area of 2,345,095 km^2 and crossed by lakes, rivers, forests (equatorial and tropical) and savannas, the Democratic Republic of Congo has several geozoological zones that are near 168 species of snakes. In addition to these reptiles, Congolese forests and savannas are home to many other poisonous animals such as scorpions, ants, bees, etc. The venoms of all these animals are rich sources of biomolecules. Currently, only 0.01% of the 40 million toxins estimated in nature are characterized [2]. But it is paradoxical to note that despite its rich biodiversity in poisonous animals, the Democratic Republic of Congo has no internal researchers interested in work on venoms. However, this sector is booming in the 21st century and offers particularly fertile opportunities for medical research and modern therapeutics. It is in this context that we are interested in studying the biochemical composition of the venoms of certain snakes, in this case Puff Adder and African Bush Viper. These two vipers are typically African [3,4] and populate the Congolese ophidian fauna. The first is terricolous but the second is arboreal. In the past, herpetologists used the morphology of snakes for their classification. But currently, we are using more and more genetic codes of these reptiles. Chemists and biochemists, for their part, try to use appropriate analytical techniques for the identification and characterization of the constituents of venoms in order to discover the molecules of interest but also to facilitate the work of the systematicians.

Keywords: Poisonous; Miniaturization; Venom

Introduction

Modern analytical techniques have developed strongly in recent decades with respect to the growth of sensor miniaturization, data digitization and computing power to enable the use of high performance mathematical tools with equipment less and less bulky [5,6].

Biochemists currently have a wide variety of techniques for analyzing biomolecules such as snake venom proteins. The frenetic research of scientists for the high precision that must characterize the results of their work always pushes them to perfect their analysis techniques on the one hand and on the other hand, to combine them for a better performance [7]. Mass spectrometry has become a powerful analytical tool over the last 20 years. The discovery of new ionization modes (Electrospray ionization (ESI), matrix-assisted desorption-ionization or MALDI), which earned their authors part of the Nobel Prize in Chemistry in 2002, made it possible to implement this family, and in almost all types of samples, and in particular to biological macromolecules [8]. In this study, we are interested in the development of an approach based on mass spectrometry to allow characterizing progressively the venoms of Congolese snakes. The results of this study will make it possible, on the one hand, to build a database of Congolese snake venom proteins and, on the other hand, to understand the differences between these two serpents in terms of the biochemical constitution of their respective venoms.

Biological material

The venoms of Puff Adder and African Bush Viper are taken manually from live specimens of the Lwiro serpentarium (South Kivu Province) in DR Congo. The venoms are then frozen and freeze-dried and stored at 4°C.

Method

Our samples were analyzed using the Waters process with the assistance of MassLynx software (c: \ masslynx \ snake venom \ snake venom.pro \ acqudb \ 20151209_mse_60min) for data processing. The details of the different steps are as follows:

Run method parameters

Pump

Waters Acquity SDS

Run Time: 60.00 min

Comment:

Solvent Selection A: A1

Solvent Name B: 0.1% FA Acetonitrile

Switch 1: No Change

Switch 2: No Change

Switch 3: No Change

Seal Wash: 20.0 min

Chart Out 1: System Pressure

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Chart Out 2: %B

System Pressure Data Channel: Yes

Flow Rate Data Channel: No

%A Data Channel: No

%B Data Channel: No

Primary A Pressure Data Channel: No

Accumulator A Pressure Data Channel: No Primary B Pressure Data Channel: No

Accumulator B Pressure Data Channel: No

Degasser Pressure Data Channel: No

Gradient Table

Time (min) Flow Rate %A %B Curve

1. Initial 0.300 98.0 2.0 Initial

2. 2.00 0.300 98.0 2.0 6

3. 40.00 0.300 50.0 50.0 6

4. 40.10 0.300 20.0 80.0 6

5. 50.00 0.300 20.0 80.0 6

6. 50.10 0.300 98.0 2.0 6

7. 60.00 0.300 98.0 2.0 6

Run Events: Yes

Gradient Start (Relative to Injection): 0 uL

Participate in pre-analysis: No

2D Repeat: No

Detector

Waters Acquity TUV

Run Time: 30.00 min

Wavelength Mode: Single Wavelength

Lamp On: On

Channel A..

Comment:

Wavelength: 205 nm

Sampling Rate: 20 points/sec

Data Mode: Absorbance

Time Constant: 0.1 sec

Auto Zero on Wavelength Change: Maintain Baseline

Auto Zero On Inject Start: Yes

Analog 1...

Sensitivity: 2.000 AUFS

Chart Polarity: Positive (+)

Voltage Offset: 0 mV

Enable Chart Mark: Yes

Run Events: Yes

Pulse Width: 1.0 sec

Rect Wave Period: 0.2 sec

Auto sampler

Waters ACQUITY FTN Auto Sampler

Run Time: 60.00 min

Comment:

Load Ahead: Disabled

Loop Offline: Automatic min

Wash Solvent Name: Water

Pre-Inject Wash Time: 0.0 sec

Post-Inject Wash Time: 6.0 sec

Purge Solvent Name: Water

Dilution: Disabled

Dilution Volume: 0 uL

Delay Time: 0 min

Dilution Needle Placement: 4 mm

Target Column Temperature: Off C

Target Sample Temperature: 8.0 C

Sample Temperature Alarm Band: Disabled

Syringe Draw Rate: Automatic

Needle Placement: Automatic

Pre-Aspirate Air Gap: Automatic

Post-Aspirate Air Gap: Automatic

Column Temperature Data Channel: No

Room Temperature Data Channel: No

Sample Temperature Data Channel: No

Sample Organizer Temperature Data Channel: No

Sample Pressure Data Channel: No

Preheater Temperature Data Channel: No

Seal Force Data Channel: No

End detector

Run Events: No

Sample Run Injection Parameter

Injection Volume (uL) - 4.00

End autosampler

End of experimental record..

Generic Instrument Postrun Report

Software Version: 1.60.2782

Firmware Version: 1.60.2274 (Aug 10 2013)

Checksum: 0xd71e0eda

Serial Number: E10TUV015A

Lamp On/Off Event: No

Lamp Life: 1744.00

Lamp Serial Number: 1F8ABD2

Flow Cell Type: Analytical LG

Flow Cell Path Length: 10.000 mm

Flow Cell Volume: 0.50 micro liters

Flow Cell Serial Number: 10346

Flow Cell Part Number: 205015016

Optics Temperature Stabilization Setting: Normal Temperature

Waters Acquity SDS Postrun Report

IcsVersion: 1.60.1872

Firmware Version: 1.60.267 (Aug 19 2013)

Checksum: 0x36f220a9

Serial Number: C14BUR100G

Minimum System Pressure: 2890.5

Maximum System Pressure: 4731.5

Average System Pressure: 4248.0

Minimum Degasser Pressure: 0.5

Maximum Degasser Pressure: 0.5

Average Degasser Pressure: 0.5

Software Version: 1.60.1774

Firmware Version: 1.60.364 (Sep 20 2013)

Checksum: 0x35d5392b

Serial Number: B14USM471G

Needle Size: 15.0

Minimum Sample Temperature: 8.0

Maximum Sample Temperature: 8.3

Average Sample Temperature: 8.2

Minimum Column Temperature: -0.2

Maximum Column Temperature: 0.0

Average Column Temperature: -0.2

Results

HRMS Analysis of samples from snake venom

This data contains an overlay of total ion chromatogram of all two samples [Figures 1 and 2].

• Peptides have eluted from ~ 6 min to ~ 35 min.

• Due to presence of high number of peptides, the TIC gives a bell-shaped curve.

Every scan of the TIC gives mass spectral information of eluted/co-eluted peptides.

- This data contains an overlay of mass spectra of combined scan [Figures 3 and 4]

At ~24 min of the mass chromatogram, of all two samples.

- Mass spectral information is obtained for peptide present

Discussion

In the present study, snake venom toxin proteins of Puff Adder and African Bush Viper were analyzed with the help of Mass Spectrometric. The venom of Puff Adder contains 44 % of proteins against 36 % for African Bush Viper. Generally, snake venoms are considered to contain mainly proteins, ranging from 70 to 90 % (10). In their study by using the two-dimensional gel electrophoresis, Vijayan J. et al were reported the protein content of eight Malaysian snake venom was between 30 and 80% (11). According the molecular weight (Mw) of proteins, the Puff Adder venom contains 15, 9% of Mw (1000-9000 Da), 68, 2% of Mw (10000-49000 Da), 15, 9% of Mw (50000-99000 Da) and 0, 0% of Mw (100000-190000 Da). However, African Bush Viper venom comprise 2,8% of Mw (1000-9000Da), 44,4% of Mw (10000-49000 Da), 27,8% of Mw (50000-99000 Da) and 25,0% of Mw (100000-190000

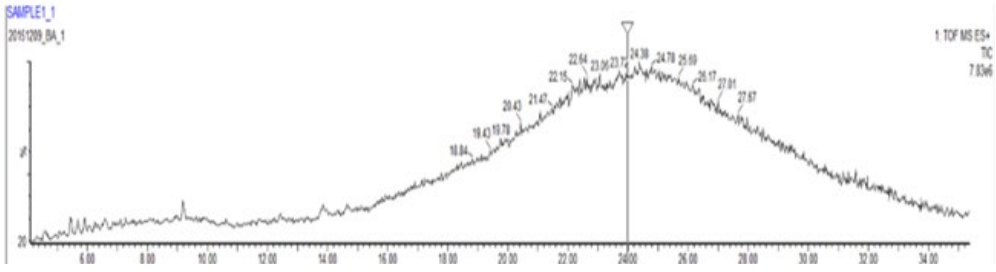

Figure 1: Mass Chromatogram - TIC of Puff Adder.

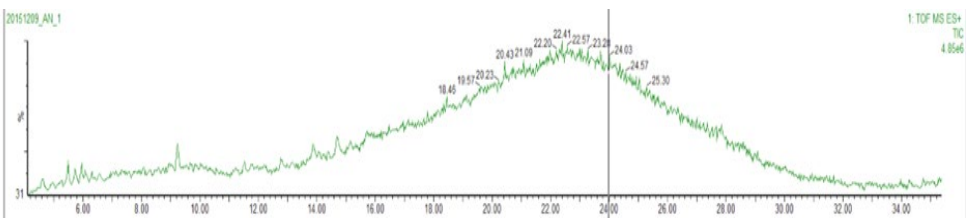

Figure 2: Mass Chromatogram - TIC of African Bush Viper.

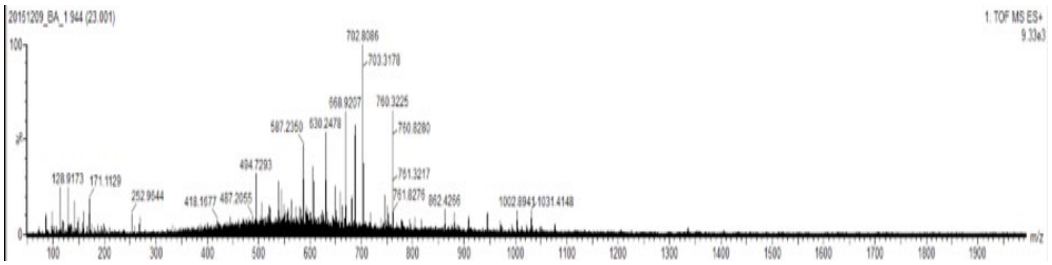

Figure 3: Mass Spectra of Puff Adder.

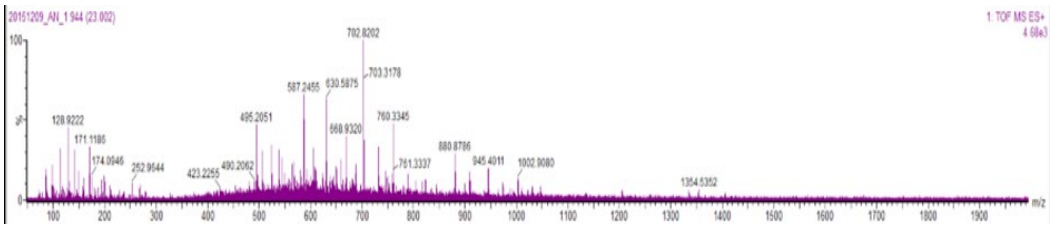

Figure 4: Mass Spectra African Bush Viper.

Da). Both venoms under study belong to the Viperidae family. Several studies show that enzymes are high molecular weight proteins [9] this is what we find in this work. It should be noted that the African Bush Viper venom contains many enzymes of very high molecular weight compared to that of Puff Adder. The hydropathy index is a measure that allows knowing the hydrophilic or hydrophobic character of a region of a protein through the amino acid sequence. A positive value of the index corresponds to a hydrophobic behavior and a negative value corresponds to a hydrophilic behavior.

The GRAVY (medium hydropathy) of these two venoms shows that 18.2% of the Puff Adder venom proteins are hydrophobic and 81.8% are hydrophobic. However, in the African Bush Viper venom, 5.6% is hydrophobic and 94.4% hydrophilic. The amino acid profiles of the venom proteins examined show that Adder Puff possesses a venom rich in negatively charged amino acids (Asp + Glu) while that of African Viper has many positively charged amino acids (Arg +

Table 1: Physicochemical characterization of venom toxin proteins of Puff Adder.

N°	Accession	Entry	Protéins	mW(Da)	pI(pH)	Peptides	AA	R^-	R^+	Gravy
1	P85062	PLA21_ACASE	Phospholipase A2 acanmyotoxin	3572	10, 1206	2	24	1	5	-0, 18
2	U3FVF0	U3FVF0_MICFL	Phospholipase A2 2d	16191	7, 5425	1	34	0	2	0, 42
3	Q7ZTA7	PA2AD_CROOA	Acidic PhospholipaseA2 coaPLA2	15538	4, 793	5	122	18	11	0,01
4	H8PG88	H8PG88_9SAUR	Phospholipase A2 isoform 7	15732	4, 812	4	106	5	6	0, 22
5	F8QN54	PA2B_VIPRE	Basic Phospholipase A2 vurtoxin	15625	8, 0127	5	65	9	13	-0, 29
6	U3FVE6	U3FVE6_MICFL	Phospholipase A2 3a	15902	8, 02	5	83	8	11	-0, 17
7	P0CV89	PA23_CROAT	Phospholipase A2	7356	8, 291	3	41	4	6	-0, 14
8	A6MFM0	A6MFM0_DEMVE	PLA2-1	17050	8, 3467	6	81	9	13	-0, 21
9	Q1PS45	VM3AK_DEIAC	Zinc metalloproteinase- disintegrin- like agkihagin	67527	5, 7202	7	106	18	15	-0, 23
10	Q90ZI3	VM3H1_PROFL	Zinc metalloproteinase- disintegrin- like HV1	68146	5, 9341	8	176	23	20	-0, 17
11	Q90WC0	VM2HS_GLOBR	Zinc metalloproteinase/ disintegrin	35086	5, 3027	4	65	11	9	-0, 27
12	Q9DGB9	VM3V1_CROAT	Zinc metalloproteinase- disintegrin- like VAP1	67915	5, 8594	9	144	28	21	-0, 28
13	V8NWK6	V8NWK6_OPHHA	Histone H3. V1	13133	4, 1382	7	107	76	20	-0, 84
14	V8N7X0	V8N7X0_OPHHA	Histone H3. V1	12391	4, 6714	5	82	55	18	-0, 83
15	V8N308	V8N308_OPHHA	Retrotransposon- like protein1	11731	3, 438	4	117	95	13	-0, 74
16	V8N872	V8N872_OPHHA	Retrotransposon- like protein1	19358	9, 4893	5	72	18	11	-0, 67
17	V8NG53	V8NG53_OPHHA	Retrotransposon- like protein1	47080	4, 5308	12	203	124	36	-0, 72
18	V8N361	V8N361_OPHHA	Uncharacterized protein	6476	4, 4033	4	57	28	8	-0, 42
19	V8PDK0	V8PDK0_OPHHA	Uncharacterized protein	11979	11, 7759	9	144	62	26	-0, 63
20	V8N2R6	V8N2R6_OPHHA	Uncharacterized protein	17675	4, 0825	10	146	103	27	-0, 92
21	V8N9J4	V8N9J4_OPHHA	Uncharacterized protein	18398	4, 6128	5	151	40	14	-0, 15
22	V8NYZ7	V8NYZ7_OPHHA	Uncharacterized protein	15650	8, 6279	4	46	15	8	-0, 35
23	V8N6V0	V8N6V0_OPHHA	Uncharacterized protein	17380	4, 8208	8	147	118	25	-0, 89
24	V8NK56	V8NK56_OPHHA	Uncharacterized protein	18367	4, 1646	10	175	133	36	-95, 46
25	V8N9X8	V8N9X8_OPHHA	Uncharacterized protein	18737	10, 4224	7	73	38	19	-0, 79
26	V8N4T1	V8N4T1_OPHHA	Uncharacterized protein	29180	6, 3296	5	83	16	10	-0, 15
27	V8NXX8	V8NXX8_OPHHA	Mnn4	21874	5, 6382	10	121	55	27	-0, 69
28	U3ESU0	U3ESU0_MICFL	Heat shock protein 90a	84071	4, 7798	8	181	47	17	-0, 14
29	V8NS42	V8NS42_OPHHA	X- linked retinitis pigmentosa GTPase regulatorinteracting protein 1	16253	3, 8569	7	112	74	18	-0, 75
30	V8N7T8	V8N7T8_OPHHA	Protein TSPEAR	32648	5, 6909	1	42	2	2	0, 27
31	R4FK10	R4FK10_9SAUR	NP-Hop-00061	12222	9,0234	1	51	2	4	0,23
32	V8NJ76	V8NJ76_OPHHA	Disabled-like 2	67633	4,9966	6	97	12	11	-0,12
33	V8NHR1	V8NHR1_OPHHA	Keratin-associated protein 16-7	16967	9,0879	6	68	2	14	-0,29
34	U3FZU3	U3FZU3_MICFL	N-Acetyltransferase 15	27544	6,2637	2	74	7	5	0,21
35	V8PHX1	V8PHX1_OPHHA	Sushi, von Willebrand factor type A, EGF and pentatraxin domain-containing protein 1	16615	7,9849	6	76	6	15	-0,27
36	T1E3X9	T1E3X9_CROHD	Metalloproteinase (Type III)	67908	5, 937	11	176	27	25	-0 ,29
37	V8N2W0	V8N2W0_OPHHA	Myelin transcriptor factor 1	5416	3, 0776	2	24	20	1	-0, 53
38	V8N4P8	V8N4P8_OPHHA	Cyclic nucleotide-gated cation channel beta-1	9931	10, 6377	6	59	24	20	-0, 99
39	A0A0B8RUP4	A0AB8RUP4_BOIIR	Epithelial membrane protein 3-like isoform 1	18671	8, 4858	3	87	2	5	0, 55
40	Q60620	Q60620_MOUSE	Cellular disintegrin- related protein	4889	4, 8516	3	43	7	7	-0, 29
41	P85040	VKTC9_DABSI	Kunitz- type serine protease inhibitor C9	2048	5, 2778	1	14	2	1	0, 18
42	A0A098LWU4	A0A098LWU4_9SAUR	3Ftx	11652	6, 0396	2	41	2	4	-0, 17
43	U3FZA8	U3FZA8_MICFL	Peripheral myelin 22- like protein 1	17807	9, 0703	2	71	0	2	0, 62
44	V8N614	V8N614_OPHHA	Phosphatidylinositol- 4,5- bisphosphate subunit	62349	6, 2241	14	247	166	55	-0, 85

Table 2: Physicochemical characterization of venom toxin proteins of African Bush Viper.

N°	Accession	Entry	Proteins	mW(Da)	pI(pH)	Peptides	AA	R^-	R^+	Gravy
1	V8NCE7	V8NCE7_OPHHA	Zinc finger CCCH domain-containing protein 13	31417	11, 3906	5	62	26	28	-1,36
2	V8NBP7	V8NBP7_OPHHA	Zinc finger CCCH domain- containing protein 13	63208	11,0068	9	89	17	33	-0, 84
3	V8P5ZO	V8P5ZO_OPHHA	Zinc finger CCCH domain-containing protein 13	58424	10,9614	2	16	6	8	-1,32
4	V8PGD1	V8PGD1_OPHHA	Zinc finger CCCH domain- containing protein 13	123264	10,4692	2	17	6	7	-1,15
5	V8N8C3	V8N8C3_OPHHA	RNA- binding protein 25	20813	10,0781	2	25	12	12	-1,58
6	V8PD92	V8PD92_OPHHA	RNA- binding protein 25	131844	10,6714	9	82	10	27	-0,69
7	V8NBD6	V8NBD6_OPHHA	RNA- binding protein 25	50011	11, 521	6	58	12	23	-0,96
8	V8P846	V8P846_OPHHA	RNA- binding protein 25	35473	10, 7842	4	34	9	14	-1,06
9	V8N352	V8N352_OPHHA	RNA- binding protein 25	40429	11, 1841	2	15	6	9	-1, 47
10	V8NUB1	V8NUB1_OPHHA	Histone- lysine N-methyltransferase, H3 lysine-79	23116	10, 8018	6	56	20	25	-1, 31
11	V8NWG1	V8NWG1_0PHHA	Histone- lysine N-methyltransferase, H3 lysine-79	40563	9, 4131	7	72	19	27	-0,93
12	V8N6E5	V8N6E5_OPHHA	Histone- lysine N-methyltransferase, H3 lysine-79	34420	10, 7798	7	55	12	22	-0, 85
13	V8NHP2	V8NHP2_OPHHA	Histone- lysine N-methyltransferase, H3 lysine-79	63735	10, 8018	9	94	30	38	-0, 96
14	V8NI47	V8NI47_OPHHA	Genetic suppressor élément 1	43705	10, 6025	2	22	5	7	-0,72
15	V8NW91	V8NW91_OPHHA	Genetic suppressor élément 1	120067	9, 0264	7	73	15	23	-0, 76
16	V8N310	V8N310_0PHHA	Uncharacterized protein	4594	11, 1753	2	16	7	9	-1, 75
17	V8NCV2	V8NCV2_0PHHA	Uncharacterized protein	91334	8, 7554	10	123	22	29	-0, 46
18	V8NFR8	V8NFR8_OPHHA	Uncharacterized protein	187479	4, 5732	8	54	7	18	-0, 60
19	V8N3P1	V8N3P1_OPHHA	Protein PRRC2C	10912	11, 5884	2	22	8	13	-1, 62
20	A0A0B8RQF8	A0A0B8RQF8_BOIIR	Protein Red-like	64671	6, 271	11	108	32	37	-0, 84
21	V8N2M1	V8N2M1_0PHHA	Claudin-19	41152	10, 7944	5	46	9	19	-0,95
22	V8NTJ4	V8NTJ4_OPHHA	Putative ATP- dépendent RNA helicase DHX30	156258	10, 2876	11	107	26	36	-0, 81
23	V8NIV6	V8NIV6_OPHHA	Agrin	54017	9, 5024	7	88	14	25	-0, 64
24	V8PDZO	V8PDZO_OPHHA	Octapeptide- repeat protein T2	48515	11, 6924	6	59	10	19	-0, 71
25	V8NDD4	V8NDD4_OPHHA	Arginine- glutamic acid dipeptide repeats protein	55363	9, 1816	5	71	12	16	-0, 41
26	V8NL62	V8NL62_OPHHA	Spore wall protein 2	80062	10, 3755	10	139	66	31	-0, 71
27	V8PDR1	V9PDR1_OPHHA	Atrophin-1	129302	9, 3677	7	82	21	25	-0, 43
28	A0A0B8RPJ1	A0A0B8RPJ1_BOIIR	Ubiquitin protein ligase E3B isoform 2	122414	8, 3965	8	79	15	17	-0, 42
29	A0A0B8RVM4	A0A0B8RVM4_ BOIIR	Tyrosine- protein kinase	50659	6, 8599	1	20	3	3	-0, 13
30	V8P5Y1	V8P5Y1_OPHHA	WD repeat- containing protein 60	114393	7, 9775	13	154	22	39	-0, 43
31	V8PB79	V8PB79_OPHHA	Cell division cycle protein 16-like protein	116253	6, 353	8	95	21	27	-0, 61
32	V8PH17	V8PH17_OPHHA	Myosin light chain 4	20837	4, 4106	1	17	5	2	-0, 07
33	V8N477	V8N477_OPHHA	Caldesmon	40823	10, 0313	10	147	53	49	-0, 93
34	V8P6C1	V8P6C1_OPHHA	5-hydroxytryptamine receptor 1D	42000	8, 5356	5	102	0	11	0, 33
35	J3S9S3	J3S9S3_CROAD	Transgelin	22150	9, 085	4	39	3	7	-0, 32
36	V8PHM9	V8PHM9_OPHHA	Ashwin	25809	8, 4624	5	60	7	14	0, 27

Lys). Lysine and arginine and their adequate presence help to become effective in the bio-molecule [10]. The most striking element in this study is that both venoms are made up of proteins of different natures. This leads some authors to talk about the variability of venoms that can be observed even within the same family. This variability of the venoms would be observed on the symptomatology related to the local necrosis for example, presented during an envenomation by these two different vipers. We also find that the African Bush Viper venom is free of phospholipase A2. However one of the most important protein super-families present in snake venoms are the phospholipasesA2 (PLA2, E.C. 3.1.1.4), a class of heat-stable and highly homologous enzymes, which catalyze the hydrolysis of the 2-acyl bond of cell membrane phospholipids releasing arachidonic acid and lysophospholipids.

Conclusion

At the end of our analysis, we realize that although both snakes

Table 3: Amino acid composition profile (in %) of Puff Adder venom toxin proteins.

N°	Accession	Ala	Cys	Asp	Glu	Phe	Gly	His	Ile	Lys	Leu	Met	Asn	Pro	Gln	Arg	Ser	Thr	Val	Trp	Tyr
1	P85062		8,3	4,1		4,1	8,3	4,1	8,3	4,1	8,3	4,1	4,1	4,1	4,1	16, 6			4,1		12, 5
2	U3FVF0	11,7	2,9			2,9	2,9	5,8	8,8	2,9	26, 4	2,9	5,8	8,8		2,9	8,8		5,8		
3	Q7ZTA7	3,2	9,8	4,0	10, 6	40	9,8	2,4	2,4	4,9	5,7	1,6	2,4	8,1	3,2	4,0	4,9	3,2	6,5	2,4	**5,7**
4	H8PG88	6,6	5,6	4,7		2,8	12, 2	2,8	1,8	2,8	16, 0	1,8	3,7	9,4	4,7	2,8	3,7	4,7	6,6	0,9	5,6
5	F8QN54	3,0	16, 9	9,2	4,6	3,0	6,1	1,5	4,6	12, 3	3,0		6,1	6,1		7,6	3,0	6,1	1,5		4,6
6	U3FVE6	7,2	12, 0	6,0	3,6	4,8	13, 2	1,2	1,2	6,0	2,4		12, 0	2,4		8,4	2,4	7,2	4,8	1,2	3,6
7	P0CV89	2,4	21, 9	4,8	4,8	7,3	9,7	2,4	2,4	4,8			2,4	2,4	2 ,4	9,7	2,4	4,8	2,4	2,4	9,7
8	A6MFM0	7,4	13, 5	8,6	2,4	1,2	12, 3	3,7	1,2	9,8	1,2	2,4	4,9	1,2	1,2	6,1	2,4	**6,1**	**1,2**	**2,4**	**9,8**
9	Q1PS45	13,2	13, 2	11, 3	5,6	2,8	6,6			8,4	2,8	4,7	2,8		7,5	5,6	3,7	7,5	1,8	0,9	0,9
10	Q90ZI3	10,7	13, 6	7,9	5,1	2,8	7,9	0,5	0,5,	4,5	2,2	2,2	7,3	3,4	5,6	6,8	3,4	5,6	6,2		2,8
11	Q90WC0	7,6	15, 3	12, 3	4,6	3,0	12, 3		3,0	4,6	4,6	1,5	4,6	1,5	9,2	9,2	3,0				3,0
12	Q9DGB9	6,2	15, 2	11, 1	8,3	2,7	7,6			6,9	1,3	2,0	5,5	2,7	2,7	7,6	3,4	6,9	6,2		2,7
13	V8NWK6				71, 0		0,9			17, 7					6,5	0,9			2,8		
14	V8N7X0				67, 0		7,3			20,7					3,6	1,2					
15	V8N308	0,8			81, 1	0,8	5,1			10, 2					0,8	0,8					
16	V8N872		4,1	11,1	13, 8		6,9		2,7	11, 1		2,7	38, 8			4,1	2,7			1,3	
17	V8NG53	0,4			61,5		20,1			6,8						10,3			0,4		
18	V8N361	7,0		21,0	28,0					14,0		14,0		7,0	1,7					7,0	
19	V8PDK0	3,4		5,5	37,5		20,1			6,9						11,1	14,5				
20	V8N2R6			2,7	67,8		4,1			6,8			0,6		3,4	11,6		0,6	2,0		
21	V8N9J4	7,2	3,3	2,6	23,8	1,9	5,9	1,3	1,3	3,9	3,3		3,3	9,2	0,6	5,2	4,6	10,5	9,9	1,3	
22	V8NYZ7	2,1	8,6	17,2	15,2	2,1	4,3		4,3	15,2	4,3	8,6			6,5	2,1	4,3	2,1		2,1	
23	V8N6V0				80,2		1,3			13,6					1,3	3,4					
24	V8NK56			2,2	73,8		1,1			18,7	0,5	0,5				1,7	1,1				
25	V8N9X8			1,3	50,6		21,9			15,0						10,9					
26	V8N4T1	13,2	4,8	7,2	12,0	1,2	14,4	1,2	1,2	3,6	7,2	2,4	3,6	3,6	1,2	8,4	4,8	4,8	2,4		2,4
27	V8NXX8	0,8		5,7	40, 4		25, 6			8,2		1,6				14, 0	2,4	0,8			
28	U3ESU0	4,9		6,0	20,4	4,9		0,5	6,6	6,0	6,0	4,4	4,9	3,3	6,6	2,7	7,7	4,4	4,4	0,5	2,7
29	V8NS42	0,8		0,8	65, 1		7,1			15, 1					8,0	0,8			0,8	0,8	
30	V8N7T8	9,5		2,3	2,3	9,5		2,3	11, 9	2,3	9,5		9,5	4,7	2,3	2,3	11, 9	7,1	7,1		4,7
31	R4FK10	15,6		1,9	1,9		11, 7		1,9	3,9	23, 5	3,9		15, 6	7,8	3,9	1,9	3,9	1,9		
32	V8NJ76	6,1		9,2	3,0	4,1	7,2		1,0	7,2	5,1	7,2	5,1	6,1	8,2	4,1	8,2	8,2	7,2		2,0
33	V8NHR1	1,4	10, 2	2,9		2,9	13, 2		1,4	5,8	2,9	1,4	1,4	8,8	2,9	14, 7	14, 7			1,4	13, 2
34	U3FZU3	4,1	1,3	6,8	2,7	4,1	10, 9	2,7	9,5	4,1	10, 9		1,3	6,8	4,1	2,7	5,4	6,8	8,2	1,3	5,4
35	V8PHX1	6,5	19, 7	6,5	1,3	1,3	6,5	2,6	1,3	14, 4	2,6		5,2		2,6	5,2	11, 8	3,9	3,9		3,9
36	T1E3X9	8,5	13, 6	10, 2	5,6	2,2	7,9	0,5		6,8	2,2	1,1	6,8	2,2	5,6	6,8	3,9	6,8	4,5		3,9
37	V8N2W0			4,1	79,1				8,3	4,1	4,1										
38	V8N4P8	1,6			40, 6		16, 9	1,6		11, 8			3,3		1,6	22, 0					
39	A0A0B8RUP4	8,1		2,3		11,6	1,1	3,4	6,9	4,6	25, 5	5,6			2,3	1,1	8,1	5,8	9,3	2,3	1,1
40	Q60620	6,9	20, 9	9,3	6,9	4,6	4,6	4,6		16, 2	4,6		13, 9				6,9				
41	P85040	14,2	14, 2	7,1	7,1	7,1	7,1			7,1	7,1	7,1		14, 2							7,1
42	A0A098LWU4	2,4	9,7	2,4	2,4	7,3	4,8		4,8	7,3	14, 6	4,8		2,4	2,4	2,4	9,7	7,3	7,3		7,3
43	U3FZA8	2,8				1,4	8,4	5,6	11, 2		28, 1	2,8	2,8		2,8	2,8	8,4	2,8	16, 9	2,8	
44	V8N614			1,6	65, 5	0,8	8,0			17, 4					1,2	4,8	0,4				

Table 4: Amino acid composition profile (in%) of African Bush Viper venom toxin proteins.

N°	Accession	Ala	Cys	Asp	Glu	Phe	Gly	His	Ile	Lys	Leu	Met	Asn	Pro	Gln	Arg	Ser	Thr	Val	Trp	Tyr
1	V8NCE7				41, 9		9,7			4,8						40, 3	1, 6	1, 6			
2	V8NBP7	7 ,7		1,1	18, 8	1,1	8,8	2,2		15, 5	1,1		2,2	4,4	4,4	21, 1	6,6	1,1	1,1	2,2	
3	V8P5ZO				37, 5		6,2			18, 7						31, 2		6,2			
4	V8PGD1	5,8	5,8		35, 2		5,8			11, 7			5,8			29, 4					
5	V8N8C3				48, 0											48, 0	4,0				
6	V8PD92	2,4	1,2	1,2	10, 9	2,4	9,7	2,4		10, 9	4,8		7,3	4,8	2,4	20, 7	8,5	3,6	4,8	1,2	
7	V8NBD6	1,7	1,7	6,8	13, 7		5,1		1,7	10, 3	5,1			6,8	3,4	29, 3	5,1	6,8	1,7		
8	V8P846				26, 4		14, 7	5,8		11, 7		2,9				29, 4	2,9	5,8			
9	V8N352				40, 0					33, 3						26, 6					
10	V8NUB1			3,5	32, 1		10, 7	1,7		5,3	3,5	1,7				39, 2	1,7				
11	V8NWG1	4,1	2,7		26, 3	1,3	15, 2	2,7		9,7		1,3	1,3			27, 7	1,3	4,1			1,3
12	V8N6E5				21, 8	1,8	9,0		1,8	20, 0	7,2		7,2	1,8		20, 0	1,8	5,4		1,8	
13	V8NHP2	2,0	2,0	6,2	25, 0	1,0	7,2	1,0		14, 5	4,1	1,0			1,0	25, 0	3,1	1,0	1,0	4,1	
14	V8NI47		4,5		22, 7	9,0	4,5		4,5		4,5				4,5	31, 8	4,5			4,5	4,5
15	V8NW91	1,3		5,4	15, 0		6,8	2,7	1,3	12, 3	5,4	1,3	2,7	6,8	4,1	19, 1	8,2	4,1	1,3		1,3
16	V8N310				43, 7											56, 3					
17	V8NCV2	5,7	2,4	2,4	15, 6	1,6	10, 6	0,8	2,4	4,9	6,5		3,2	2,4	4,2	18, 8	5,7	4,9	5,7	0,8	1,6
18	V8NFR8	3,7		1,8	11, 1	9,2			3,7	22, 2		3,7	7,4	5,5	7,4	11, 1	3,7	3,7	3,7		
19	V8N3P1				36, 4					18, 2					4,5	40, 9					
20	A0A0B8RQF8	2,7		4,6	25, 0	4,6	1,8	0,9	3,7	8,3	5,5	3,7	3,7	1,8	2,7	25, 9		1,8			2,7
21	V8N2M1		2,1	4,2	17, 0		12, 7			14, 8	4,5		6,3	4,2	2,1	25, 5	2,1		2,1	2,1	
22	V8NTJ4	0,9		4,5	18, 9	0,9	1,8		2,7	6,3	13, 5	1,8	1,8	3,6	3,6	26, 1	5,4	4,5	0,9	0,9	1,8
23	V8NIV6	4,3			15, 2	3,2	6,5	1,0	2,1	7,6	7,6		5,4	7,6	5,4	19, 5	8,6	2,1	2,1	1,0	
24	V8PDZO	5,0		1,6	15, 2	1,6	22, 0	3,3	1,6	5,0	3,3	1,6	3,3		1,6	27, 1	5,0			1,6	
25	V8NDD4	9,8		4,2	12, 6		2,8	2,8	1,4	5,8	12, 6	2,8	1,4	7,0	1,4	16, 9	12, 6	1,4	2,8		1,4
26	V8NL62			2,1	45, 3		12, 9		1,4	9,3	3,5	1,4	0,7	2,8	1,4	12, 9	2,8	2,8			
27	V8PDR1	6,0		7,3	18, 2		4,8	1,2	1,2	9,7	7,3	2,4	1,2	3,6	1,2	20, 7	8,5	3,6	1,2		1,2
28	A0A0B8RPJ1	5,0		8,8	12, 6		2,5		3,7	12, 6	13, 9	2,5	3,7	1,2	5,0	12, 6	6,3	5,0	2,5		1,2
29	A0A0B8RVM4	5,0		10, 0	5,0	5,0	10,0			15, 0	10, 0				5,0		15, 0	15, 0	5,0		
30	V8P5Y1	2,5	0,6	4,4	9,5	1,9	5,0	4,4	4,4	15, 9	4,4	0,6	3,8	3,1	6,3	8,9	7,6	5,0	7,6	0,6	2,5
31	V8PB79	2,1	1,0	5,2	16, 8	2,1		2,1		15, 7	7,3	3,1	2,1	5,2	4,2	12, 6	7,3	5,2	4,2		3,1
32	V8PH17	5,8		5,8	23, 5	11,7			11, 7	11, 7					5,8		11,7	5,8	5,8		
33	V8N477	2,6	0,6	4,0	31, 3	0,6	12, 6		0,6	9,3	4,0		1,3	2,6	4,6	23, 3	1,3				0,6
34	V8P6C1	8,6	0,8	3,4	0,8	6,9	3,4	0,8	13, 0	6,0	13, 9	3,4	5,2	5,2	0,8	5,2	6,0	7,8	4,3	1,7	1,7
35	J3S9S3	10,0		2,5	5,0	2,5	12, 5		2,5	7,5	5,0	5,0	7,5	5,0	7,5	12, 5	5,0		2,5	2,5	5,0
36	V8PHM9	7,0	2,8	7,0	2,8	4,2	7,0	2,8	5,8	9,8	9,8	4,2	2,8	2,8	4,2	9,8	5,6	8,4	1,4		1,4

belong to the same family of vipers, they have fundamentally different venoms from the point of view of their biochemical compositions. This leads specialists to talk about the variability of venom. This variability is genetic, that is, specific to each individual. The variations concern both the concentration of the different fractions and their biochemical structures [11]. Mass spectrometry has been an undeniable aid to us. This allowed us to scrutinize the secrets of the venoms of these two snakes. As our samples came from the eastern part of the country, it perhaps would be useful to extend this study to all geozoological areas of the Democratic Republic of Congo but also to other venomous species that this vast territory has.

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