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ISOLATION, CLONING AND CHARACTERIZATION OF *POLISTES DOMINULUS* VENOM PHOSPHOLIPASE A1 AND ITS ISOFORMS

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Polistes dominulus is known to be an allergenically important social wasp. Its venom has four major allergens (*ziv*, phospholipase A1, hyaluronidase, antigen 5 and a serine-protease). Amino acid sequences of its serine-protease and Antigen 5 have been published. In this paper, the partial amino acid sequence of its venom phospholipase native protein is reported. Also, we give an account to the complete nucleotide sequence of *Polistes dominulus* venom phospholipase A1 gene, its isoforms and their complete deduced amino acid sequences. Their similarity to the other phospholipases A1 of the family: Vespidae is discussed.

Keywords: Hymenoptera – Vespidae – Polistinae – *Polistes dominulus* – Venom phospholipase A 1.

INTRODUCTION

Many Hymenoptera species are capable of stinging, but only species belonging to three families sting people commonly and cause insect sting-allergic reactions [15]. These are Apidae (bees), Formicidae (ants) and Vespidae (wasps) [13, 9]. *Polistes dominulus* (Hymenoptera: Vespidae; Polistinae) is one of the medically important species [6]. A case of death after anaphylactic reaction to a vespid was reported [4]. The clinical history of the patient, RAST and the RAST-Inhibition results and the geographical distribution of the vespids indicate that the responsible species was "*Polistes dominulus*". *Polistes dominulus*, the yellow jacket *Vespula germanica* and the hornet *Vespa crabro* are known to be allergenically important social wasps found in southern Europe [21] and the mediterranean region. Different species of Vespidae inhabit different areas of the United States. *Polistes dominulus* is one of these species [14] introduced into the northeast, USA.

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Abbreviations: aa. no.: amino acid number; nt. no.: nucleotide number; PCR: Polymerase Chain Reaction; SMART: Switching Mechanism At 5' end of RNA Transcript; RACE: 5'- and 3'-Rapid Amplification of cDNA Ends; RAST: Radioallergosorbent test

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The importance of venoms as allergens has been known for some time [17, 16]. Vespidae venoms have similar venom compositions and their major venom allergens are phospholipase A1, hyaluronidase and antigen 5 a protein of unknown biological function [12, 13]. A fourth allergen, a serine-protease has been detected in *P. exclamationis*, *P. dominulus* and *P. gallicus* venoms [10, 18, 20].

Amino acid sequences of two of four major *Polistes dominulus* venom allergens have been reported; the serine-protease [5] and Antigen 5 [8]. In this paper, we report partial amino acid sequence of *Polistes dominulus* venom phospholipase native protein and complete nucleotide sequence of its gene and its complete deduced amino acid sequence.

MATERIALS AND METHODS

Venom

Commercial grade venom sac extract was obtained from Vespa Laboratories (Spring Mills, Pa.). Protein components of the venom were purified on Sephadex G50 using 0.1 M NH_4HCO_2 (pH 5.0), 0.15 M NaCl. The second peak exhibited phospholipase activity when it tested as described [7]. This fraction was further purified using Mono S high-performance cation exchange chromatography. The fraction eluted at 33–35 minutes was run on a Vydac C4 column with monitoring at 220 nm. N-terminal sequencing for the fraction eluted at 61.5 minutes was carried out as described below.

Reduction and alkylation

Purified proteins were reduced in 8 M ultrapure urea plus TRIZMA pH 7.4 (Sigma[®]) up to 0.2 M, then 5 mg/ml dithiothreitol was dissolved and purged with argon. For each 0.1–1 mg dried protein 0.4 ml of dithiothreitol (DTT) was added, layered with argon and heated to 56 °C for 1 hour. The reduced proteins were alkylated with 50 μl iodoacetic acid solution [108 mg iodoacetic acid (Pierce Chemical Co., Rockford, Ill.), freshly dissolved in 1.0 ml of 0.5 N sodium hydroxide], in the dark under argon at room temperature for 30 minutes. Denaturing agents and excess reactants were removed by dialysis against 0.04 M ammonium bicarbonate (pH 8.0) using a Pierce Slide-A-Lyzer cassette (Pierce Chemical Co., Rockford, Ill.).

Protein digestion

Overnight trypsin digestion of the alkylated protein was carried out at 37 °C with continuous stirring. For each 100 μl protein, 1 μl of 0.1 M calcium chloride and 8 μg of Promega modified trypsin were added. Staphylococcal endoproteinase-glutamic

Amino acid sequence of

acid-C (V8) protease
chased from Pierce
Enzyme hydrolyses
Co.) to a final concn

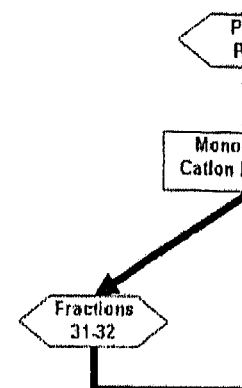


Fig. 1. Flow chart

a for some time [17, 16].
r major venom allergens
in of unknown biological
been detected in *P. excla-*

minulus venom allergens
]. In this paper, we report
phospholipase native pro-
complete deduced amino

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aspa Laboratories (Spring
l on Sephadex G50 using
exhibited phospholipase
ther purified using Mono
fraction eluted at 33–35
g at 220 nm. N-terminal
d out as described below.

RIZMA pH 7.4 (Sigma®)
d purged with argon. For
was added, layered with
were alkylated with 50 µl
Chemical Co., Rockford,
], in the dark under argon
d excess reactants were
e (pH 8.0) using a Pierce
).

arried out at 37 °C with
alcium chloride and 8 µg
endoproteinase-glutamic

acid-C (V8) protease digestion was performed with the insolubilized enzyme kit purchased from Pierce Chemical Co. by following the manufacturer's instructions. Enzyme hydrolyses were stopped by adding trifluoroacetic acid (Pierce Chemical Co.) to a final concentration of 0.2–0.5%.

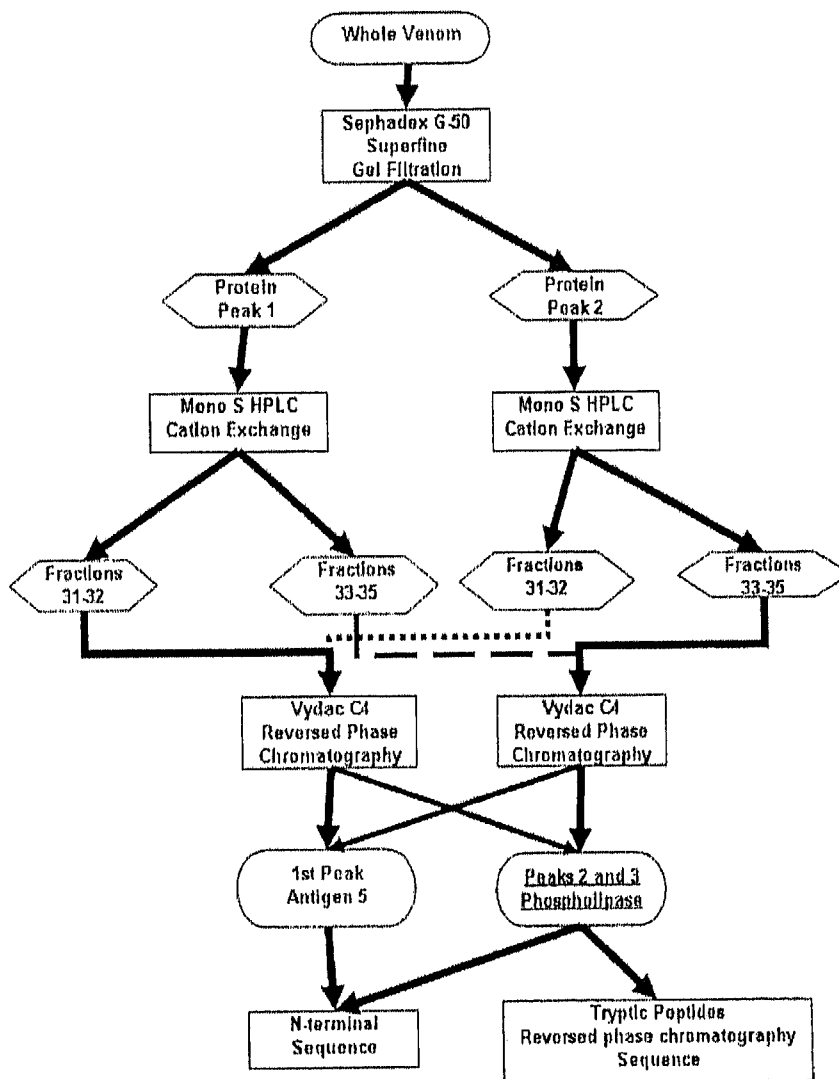


Fig. 1. Flow chart summarizes the steps of *Polistes dominulus* venom protein isolation

mRNA isolation and first strand cDNA synthesis

mRNA was isolated from the last two abdominal segments of *Polistes dominulus* using the PolyATtract[®] System 1000 kit (Promega Co.) following the manufacturer's technical manual. Five hundred ng mRNA were used in the first strand cDNA synthesis utilizing SUPERScript[™]II RNase H⁻ Reverse Transcriptase (GIBCOBRL Life Technologies, Inc.) and the following primer: 5' TCT GAA TTC TCG AGT CGA CAT CTT TTT TTT TTT TT 3'. mRNA complementary to the cDNA was removed by adding 2 U of *E. coli* RNase H and incubated at 37 °C for 20 min.

Nested PCR

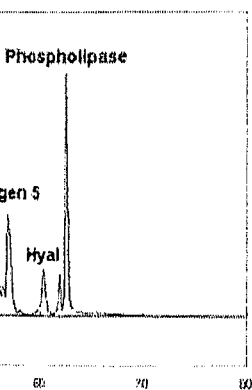
Two degenerate primers were designed based on the partial amino acid sequence obtained from the venom phospholipase protein sequencing. The forward primer (Forward): 5' TTY AAY GAR AAR GAY ATH GA 3' which encodes the amino acid motif 43 FNEKDIE 49. The reverse primer (Reverse2): 5' CCY TCR TTR TGR CAR TAN GG 3' which encodes the amino acid motif 328 PYCHNEG 333. The polymerase chain reaction was carried out using Opti-Prime™ PCR Optimization Kit (Stratagene Cloning Systems, La Jolla, CA.). Polymerase Chain Reaction carried out using The MiniCycler™ Programmable Thermal Controller model PTC-150. The PCR product was purified using QIAGEN MinElute PCR Purification Kit. The purified PCR product was cloned in pCR® 4-TOPO® vector using TOPO TA cloning® Kit for sequencing (Invitrogen™ life technologies). Plasmid DNA was isolated and purified using QIAprep Spin Miniprep Kit (QIAGEN).

3' RACE PCR

Cloning of the 3' end of clone TP1againC6

First strand cDNA synthesis was done as described before using the same primer: 5' TCT GAA TTC TCG AGT CGA CAT CTT TTT TTT TT 3'. Amplification of 3' end of clone TP1againC6 was carried out using the following primers: primer 182: 5' TCT GAA TTC TCG AGT CGA CAT CT 3' which correspond to the first 23 nucleotides of the primer used in the synthesis of first strand cDNA and primer 556Forward: 5' ATA CAT ACA TCA GCC ATA TTA GG 3' which encodes the amino acid motif 228 IHTSAILG 235 utilizing Opti-Prime™ PCR Optimization Kit (Stratagene Cloning Systems, La Jolla, CA.). Purification and cloning of the PCR product and isolation of the plasmid cDNA were done as described above.

18 column using a gradient of acetic acid in acetonitrile detected by hand using silicagel plates. The separation was done as part of the protein isolation procedure.



by Vydac C4 reversed

model 6625 Prosequencer
1-phase fully automated
coupled to either diisothio-
protein was reduced and
five times each in water
sts were coupled to ary-
re coupled to diisothio-
Acid hydrolysis was per-
tris trifluoroacetic acid at
disk, the peptides with N-
trotbenzene sulfonic acid

5' RACE PCR

Cloning of the 5' end of clone TP1againC6

First strand cDNA synthesis was carried out using BD SMART™ RACE cDNA Amplification Kit (BD Biosciences Clontech, Palo Alto, CA.). Amplification of the 5' end of clone TP1againC6 was done using the following two primers: Universal Primer A Mix (UPM) supplied with the BD SMART™ RACE cDNA Amplification Kit (BD Biosciences Clontech, Palo Alto, CA.) and primer 123Reverse 5': 5' AGG AAA CCA TGT ATA AGA ATT GC 3' which represents the amino acid motif 84 AILIHGFL 91 using Opti-Prime™ PCR Optimization Kit (Stratagene Cloning Systems, La Jolla, CA.). Purification and cloning of the PCR product and isolation of the plasmid cDNA were done as described above.

Asymmetric PCR

To explore the possibility of the presence of other isoform(s) or polymorphism asymmetric PCR was performed and followed by a single denaturation-extension cycle to eliminate the possibility of heteroduplex formation as described [2]. The following primers were used; primer 5' (22-28): 5' GCT GAT GAT TTG ACC ACC CTG 3' which encodes the amino acids motif 22 ADDLTTL 28, primer 5'+1 (22-27): 5' GAG GAG GCT GAT GAT TTG ACC ACC 3' which represents the amino acid motif 22 ADDLTT 27 and the hexanucleotide GAG GAG was added at its 5' end and primer 3' (331-337): 5' CAG TTT AAT GCC TTC GTT ATG 3' which encodes for the amino acid motif 331 HNEGILK 337.

DNA sequencing and analysis of the sequencing data

Plasmids containing cDNA inserts were sequenced using ABI automated sequencer (Applied Biosystems, Foster City, CA.) at the Molecular Biology Research Facility, University of Tennessee, Knoxville. For clones TP1againC6 and TM5'10C1 the universal primers T3 & T7 were used. For clone TM3'10C7 the primer 182 and primer 556Forward were used. For clone TMS2.1C3 the universal primers T3 & T7 were used. An internal primer, primer ForwardC3a178: 5' CAT ACT TCA GGT TTT GCA GG 3' which encodes the amino acid motif 178 HTSGFAG 184, was designed and used to verify the 3' end sequence of clone TMS2.1C3. The universal primers T3, T7 and M13 reverse were used to sequence clone TMS2.2C5. For clone TMS2.2C14 the universal primer T7 and primer ForwardC3a157 were used. For clone TMS2.2C10 the universal primer T7 and primer 123REVERSE 5' were used.

The sequencing data were analyzed using the bioinformatics packages available through the ExPASy web site (<http://us.expasy.org>) and the Biology WorkBench web site (<http://workbench.sdsc.edu/>).

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Amino acid sequence of wasp

The signal peptide sequence cleavage site next to the molecular weight of the mature are available through the AATAAA of the polyadenylation global alignment of two were done using ALIGN through Biology WorkBench.

R

Based on the partial amino acid sequence of the phospholipase (Fig. 3) two degenerate primers were used in 3' and 5' ends of the amplification of 436 residues, which has a 3' end. The 5' RACE PCR results in the amplification of 436 residues encoding for 90 amino acids (Viz. TM5'10C1). The overall length of the cDNA of 1171 base pairs, open reading frame of 1011 residues from nucleotide number 55 to 1561, which is 106 nucleotides. The overall length of 106 residues and a propeptide of the polyadenylation signal.

```

G I T P D C T F N E
X X X X N Y D L F C
X X X X X X X X X A
A F A S T N D V L C
L L V E Q Y K V P N
E V Q R X X L X K Y
X X X X X X X X X X
X X X X X X S Q P C
R E C C L I G T P W
V G L N A Q S Y P A
K L

```

Fig. 3. The partial amino acid sequence of the phospholipase.

SMARTTM RACE cDNA
(CA.). Amplification of the
using two primers: Universal
RACE cDNA Amplification
for 123Reverse 5': 5' AGG
is the amino acid motif 84
Kit (Stratagene Cloning
PCR product and isolation

s) or polymorphism asym-
metrization-extension cycle to
described [2]. The following
TTG ACC ACC CTG 3'
primer 5'+1 (22-27): 5'
represents the amino acid
was added at its 5' end and
ATG 3' which encodes for

Sequencing data

ABI automated sequencer
Biology Research Facility,
6 and TM5'10C1 the uni-
the primer 182 and primer
al primers T3 & T7 were
T ACT TCA GGT TTT
SGFAG 184, was design
3. The universal primers
e TMS2.2C5. For clone
dC3a157 were used. For
REVERSE 5' were used.
matics packages available
Biology WorkBench web

The signal peptide sequence was determined using SignalP V1.1 [19]. The trypsin cleavage site next to the propeptide was determined using PeptideCutter. The molecular weight of the mature enzyme was calculated using ProtParam tool. These tools are available through the ExPASy web site (<http://us.expasy.org>). The hexanucleotide AATAAA of the polyadenylation signal determined as described [24, 23]. Optimal global alignment of two protein sequences and global multiple sequence alignment were done using ALIGN and CLUSTALW, respectively. These tools are available through Biology WorkBench (<http://workbench.sdsc.edu/>).

RESULTS AND DISCUSSION

Based on the partial amino acid sequence of *Polistes dominulus* venom native phospholipase (Fig. 3) two degenerate primers, Forward and Reverse2, were synthesized. The PCR reaction primed using these two primers resulted in the amplification of a 875 base pair cDNA fragment which encodes for 291 amino acid residues (Viz. TP1againC6). The nucleotide sequence of this fragment allowed the synthesis of two non-degenerate primers, 556Forward and 123Reverse 5', these two gene specific primers were used in 3' and 5' RACE PCR reactions. The 3' RACE PCR resulted in the amplification of 436 base pair cDNA fragment encoding for 110 amino acid residues, which has a 3' non-coding sequence of 106 base pairs (Viz. TM3'10C7). The 5' RACE PCR resulted in the amplification of a 337 base pair cDNA fragment encoding for 90 amino acid residues with a 5' non-coding sequence of 54 base pairs (Viz. TM5'10C1). The overlapping combined data of these three clones yielded a cDNA of 1171 base pairs. This cDNA has a leader sequence of 54 nucleotides, an open reading frame of 1011 nucleotides which encodes for 337 amino acids, starting from nucleotide number 55 to nucleotide number 1065 and a 3' non-coding sequence of 106 nucleotides. The open reading frame has a signal peptide of 21 amino acid residues and a propeptide of 14 amino acid residues. The hexanucleotide AATAAA of the polyadenylation signal begins at nucleotide number 1095. The Poly (A) site

```
G I T P D C T F N E K D I E L H V Y S R D K R X G I I L K X
X X X X N Y D L F Q K S X I X H Q X X X L I H G F L X X X X
X X X X X X X X X A L I E I D N F L V I S V D W X X G A C N
A F A S T N D V L G Y S Q A V G N T R H V G K Y V A D F T K
L L V E Q Y K V P M S N I R L I G H S L G A H T S G F A G K
E V Q R X X L X K Y K E I I G L D P A G P S F L T S K X X X
X X X X X X X X X X X X X X X X X X X X X X X X X X X
X X X X X X S Q P G C S E P S C S H T K X X X Y L T E C I K
R E C C L I G T P W K S Y F S T P K S I S Q C K X D T C V C
V G L N A Q S Y P A K G S F Y V P V E K D A P Y C H N E G I
K L
```

Fig. 3. The partial amino acid sequence of *Polistes dominulus* native venom phospholipase obtained by amino acid sequencing

begins at position 1118 followed by a Poly (A) tail of 54 nucleotides. This cDNA is given the name *Polistes dominulus* venom Phospholipase A1 1 precursor (Allergen Pol d 1.01) (Fig. 4) (Genbank Accession number AY566645). Its mature enzyme has a calculated molecular weight of 33502.3 Dalton. It has 75.4%, 51.7%, 49.4%,

AAT ATT TTC AAA CCC GGA TGA TTT TAT TTC CAC AGT AAA GAA AAA TCA GAA AGG	0
M N F K Y S I L F I C F V K V L D N	18
ATG AAT TTC AAA TAT TCG ATT TTA TTC ATA TGT TTT GTG AAA GTG TTA GAT AAT	108
C Y A A D D L T T L R N G T L D R I G	36
TGT TAC GCG GCT GAT GAT TTG ACC ACC CTG AGA AAT GGT ACC TTG GAC AGA GGC	162
I T P D C T F N E K D I E L H V Y T S	54
ATC ACT CCG GAT TGT ACT TTT AAT GAG AAA GAT ATT GAG CTA CAC GTT TAC TCA	216
R D K R N G I T L K K E I L K N Y D	72
AGG GAT AAA CGA AAT GGT ATT ATT CTT AAG AAA GAA ATT TTA AAG AAT TAC GAT	270
L F Q K A A T C A A T A T C A C A A A T T G C A I L I A H G F	90
CTG TTT CAA AAA TCT CAA ATA TCA CAT CAA ATT GCA ATT CTT ATA CAT GGT TTC	324
L T S A T G G N N E A N F D A M A K A C C T L T A E	108
CTT TCA ACT GGG AAT AAC GAA AAT TTC GAT GCA ATG GCG AAA CCT TTG ATA GAA	378
I D N F L V I S V D W K K G A C T G C N A G C I	126
ATA GAT AAT TTT CTT GTA ATT TCG GTC GAC TGG AAG AAG GGT GCT TGC AAT GCT	432
F A T N D V S Q A V G N A C T R	144
TTT GCT TCA ACT AAT GAT GTT TTG GGT TAC TCC CAA GCC GTT GGA AAC ACA CGT	486
H V G A K Y V A D F T K L L V E Q Y K	162
CAC GTT GGA AAA TAT GTA GCT GAT TTT ACA AAA TTA TTA GTA GAA CAA TAT AAA	540
V P M S N I R L I G H S L G A C A T A C T T C A	180
GTG CCG ATG TCG AAT ATA CGA TTG ATC GGC CAT AGT TTG GGT GCG CAT ACT TCA	594
G F A G A K A E V Q R L K A L G A K Y K A G E I	198
GGT TTT GCA GGA AAA GAA GTT CAA AGG TTA AAA TTA GGA AAA TAT AAG AAT ATA	648
I G L D P A G P S F L T N K C P N R	216
ATC GGG CTT GAT CCT GCT GGA CCG TCT TTT CTA ACG AAT AAA TGT CCA AAC AGA	702
L E T V D A Y V Q A I H T A T C A G C C A A T A L T T A	234
CTT TGC GAA ACA GAC GCA GAA TAT GTT CAA GCT ATA CAT ACA TCA GCC ATA TTA	756
G V Y T N N V G T G G T A G C V D F T Y V N Y G K S	252
GGA GTA TAT TAT AAT GAT GGT AGC GTT GAT TTT TAC GTG AAT TAT GGA AAA AGT	810
O P G C S E C A S C T H T K A A G C C V A A A T A C T G	270
CAA CCT GGT TGC AGC GAA CCA TCC TGT TCT CAT ACG AAA GCC GTG AAA TAC CTG	864
T A C I K R E C L I G A C A P W K A G S Y	288
ACT GAG TGT ATA AAA CGT GAA TGT TGT TTA ATT GGA ACA CCA TGG AAG AGT TAT	918
F S T P K A P I S Q C K R D T C V C V	306
TTT AGC ACG CCA AAA CCA ATT TCT CAG TGC AAA AGA GAC ACC TGT GTT TGC GTT	972
G L N A Q S Y P A K G S F Y V P V D	324
GGA CTG AAC GCT CAA AGT TAT CCT GCT AAA GGC TCA TTT TAT GTA CCT GTT GAT	1026
K D A P Y C H N E G I K L * A T T G T A A A C A A T A	337
AAA GAT GCA CCT TAT TGC CAT AAC GAA GGC ATT AAA CTG TAA TTG TAA ACA ATA	1080
GAA AAT GTA TAA ATA ATA AAT GAA TAA ATG AAC AGT CAA AAA AAA AAA AAA	1134
AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA A	1171

Fig. 4. Complete nucleotide sequence and deduced amino acid sequence of *Polistes dominulus* phospholipase A1 1 (allergen Pol d 1.01). Open reading frame of 1011 nucleotides encodes for 337 amino acids, started from nt. no. 55 to nt. no. 1065. TAA Stop codon begins at nt. no. 1066. Signal peptide of 21 aa. (single underline) from aa. no. 1 to aa. no. 21. Signal peptide predicted cleavage site between position 21 and 22 indicated by arrow head (▼). Propeptide of 14 aa. (double underline) from aa. no. 22 to aa. no. 35. Trypsin cleavage site between aa. no. 35 and aa. no. 36 indicated by vertical arrow facing down. The hexanucleotide AATAAA of the polyadenylation signal begins at nt. no. 1095 (bold face). Poly (A) site begins at position 1118 followed by Poly (A) tail of 54 nucleotides

G I T P D C	GGC ATC ACT CCG GAT TGT A
S R D K E N	TCA ACG GAT AAA CGA AAT G
D L F Q K S	GAT CTG TTT CAA AAA TCT C
F I S T G N	TTC CTG TCA ACT GGG AAT A
E I D N F L	GAA ATA GAT AAT TTT CTT G
A F A S T N	GCT TTT GCT TCA ACT AAT G
R H V G K Y	CCT CAC GTT GGA AAA TAT G
K V P M S N	AAA GTG CCG ATG TCG AAT A
S G F A G A	TCA GGT TTT TCA GGA AAA G
I I G L D F	ATA ATC GGG CTT GAT CCT G
R L C E T A	AGA TTG TGC GAA ACA GAC G
L G V Y Y N	TTA GGA GTA TAT TAT AAC G
S Q P C C	AGT CAA CCT GGT GGC AGC G
L T E C I K	CTG ACT GAG TGT ATA AAA C
V F S T P K	TAT TTT AGC ACG CCA AAA C
V G L N A Q	GTT GGA CTG AAC GCT CTA A
D K D A P Y	GAT AAA GAT GCA CCT TAT T

Fig. 5. The nucleotide sequence of

47.9% and 46.8% identity with *Dolichovespula maculata* phospholipase A1, respectively.

The product of the asymmetric screening of the resulting cDNA TMS2.2C5, TMS2.2C10 and different isoforms of *Polistes* TMS2.1C3, TMS2.2C10 and venom phospholipase A1 2, phospholipase A1 3 (allergen Pol d 1.04), respectively, are not shown but they are accession numbers AY566647 and AY566649. The molecular weights of 33516.4, 33476.3 and 334

nucleotides. This cDNA is
e A1 1 precursor (Allergen
45). Its mature enzyme has
75.4%, 51.7%, 49.4%,

A	AAA	TCA	GAA	AGG	54
V	L	D	N		18
A	GTG	TTA	GAT	AAT	108
L	D	R	G		36
C	TTG	GAC	AGA	GGC	162
H	V	Y	S		54
A	CAC	GTT	TAC	TCA	216
K	N	Y	D		72
A	AAG	AAT	TAC	GAT	270
I	H	G	F		90
T	ATA	CAT	GGT	TTC	324
A	LT	I	E		108
A	GCT	TTG	ATA	GAA	378
A	C	N	A		126
T	GCT	TGC	AAT	GCT	432
G	N	T	R		144
T	GGA	AAC	ACA	CGT	486
E	Q	Y	K		162
A	GAA	CAA	TAT	AAA	540
A	H	T	S		180
T	CGC	CAT	ACT	TCA	594
Y	K	E	I		198
A	TAT	AAG	GAA	ATA	648
C	P	N	R		216
A	TGT	CCA	AAC	AGA	702
S	A	I	L		234
A	TCA	GCC	ATA	TTA	756
Y	G	K	S		252
T	TAT	GGA	AAA	AGT	810
V	K	Y	L		270
C	GTG	AAA	TAC	CTG	864
W	K	S	Y		288
A	TGG	AAG	AGT	TAT	918
C	V	C	V		306
T	TGT	GTT	TGC	GTT	972
V	P	V	D		324
T	GTA	CCT	GTT	GAT	1026
TTG	TAA	ACA	ATA		337
AAA	AAA	AAA	AAA		1134
					1171

ence of *Polistes dominulus* phos-
leotides encodes for 337 amino
at nt. no. 1066. Signal peptide of
dicted cleavage site between posi-
ble underline) from aa. no. 22 to
licated by vertical arrow facing
s at nt. no. 1095 (bold face). Poly
of 54 nucleotides

G	I	T	P	D	C	T	F	N	E	K	G	I	E	L	H	V	Y	18
GGC	ATC	ACT	CCG	GAT	TGT	ACT	TTT	AAT	GAG	AAA	GGT	ATT	GAG	CTA	CAC	GTT	TAC	54
S	R	D	K	R	N	G	I	I	L	K	K	E	I	L	K	N	Y	36
TCA	AGG	GAT	AAA	CGA	AAT	GGT	ATT	ATT	CTT	AAG	AAA	GAA	ATT	TTA	AAG	AAT	TAC	108
D	L	F	Q	K	S	Q	I	S	H	Q	I	A	I	L	I	H	G	54
GAT	CTG	TTT	CAA	AAA	TCT	CAA	ATA	TCA	CAT	CAA	ATT	CCA	ATT	CTT	ATA	CAT	GGT	162
F	L	S	T	G	N	N	E	N	F	D	A	M	A	K	A	L	I	72
TTC	CTT	TCA	ACT	GGG	AAT	AAC	GAA	AAT	TTC	GAT	GCA	ATG	GCG	AAA	GCT	TTG	ATA	216
E	I	D	N	F	L	V	I	S	V	D	W	K	K	G	A	C	N	90
GAA	ATA	GAT	AAT	TCT	CTT	GTA	ATT	TCG	GTC	GAC	TGG	AAG	AAG	GGT	GCT	TGC	AAT	270
A	F	A	S	T	N	D	V	L	G	Y	S	Q	A	V	Q	N	T	108
GCT	TTT	GCT	TCA	ACT	AAT	GAT	GTT	TTG	GGT	TAC	TCC	CAA	GCC	GTT	GGA	AAC	ACA	324
R	H	V	G	K	Y	V	A	D	F	I	K	L	L	V	E	Q	Y	126
CGT	CAC	GTT	GGA	AAA	TAT	GTA	GCT	GAT	TTT	ATA	AAA	TTA	TTA	GTA	GAA	CAT	TAT	378
K	V	P	M	S	N	I	R	L	I	G	H	S	L	G	A	H	T	144
AAA	GTG	CCG	ATG	TCG	AAT	ATA	CGA	TTG	ATC	GGC	CAT	AGT	TTG	GGT	GCG	CAT	ACT	432
S	G	F	A	G	K	E	V	Q	R	L	K	L	G	K	Y	K	E	162
TCA	GGT	TTT	GCA	GGA	AAA	GAA	GTT	CAA	AGG	TTA	AAA	TTA	GGA	AAA	TAT	AAG	GAA	486
I	I	G	L	D	P	A	G	P	S	F	L	T	S	A	C	P	D	180
ATA	ATC	GGG	CTT	GAT	CCT	GCT	GGA	CCG	TCT	TTT	CTA	ACG	AGT	AAA	TGT	CCA	GAT	540
R	L	C	E	T	D	A	E	Y	V	Q	A	I	H	T	S	A	I	198
AGA	TTG	TGC	GAA	ACA	GAC	GCA	GAA	TAT	GTT	CAA	GCT	ATA	CAT	ACA	TCA	GCC	ATA	594
L	G	V	Y	N	V	G	S	V	D	P	Y	V	N	Y	G	K		216
TTA	GGA	GTA	TAT	TAT	AAC	GTT	GGT	AGC	GTT	GAT	TTT	TAC	GTG	AAT	TAT	GGA	AAA	648
S	Q	P	S	E	P	S	C	S	H	T	C	K	A	V	K	Y		234
AGT	CAA	CCT	GGT	TGC	AGC	GAA	CCA	TCC	TGT	TCT	CAT	ACG	AAA	GCC	GTG	AAA	TAC	702
L	T	E	C	I	K	R	E	C	C	L	I	G	T	P	W	K	S	252
CTG	ACT	GAG	TGT	ATA	AAA	CGT	GAA	TGT	TGT	TTA	ATT	GGA	ACA	CCA	TGG	AAG	AGT	756
Y	F	S	T	P	K	P	I	S	Q	C	R	D	T	C	V	C		270
TAT	TTT	AGC	ACG	CCA	AAA	CCA	ATT	TCT	CAG	TGC	AAA	AGA	GAC	ACC	TGT	GTT	TGC	810
V	G	L	N	A	Q	S	T	P	A	K	G	S	F	Y	V	P	V	288
GTT	GGA	CTG	AAC	GCT	CAA	AGT	TAT	CCT	GCT	AAA	GCG	TCA	TTT	TAT	GTA	CCT	GTT	864
D	K	D	A	P	Y	C	H	N	E	G	I	K	L					302
GAT	AAA	GAT	GCA	CCT	TAT	TGC	CAT	AAC	GAA	GCG	ATT	AAA	CTG					906

Fig. 5. The nucleotide sequence and the deduced amino acid sequence of *Polistes dominulus* venom phospholipase A1 clone TMS2.2C5

47.9% and 46.8% identity with *Polistes annularis* phospholipase A1, *Vespa vulgaris* phospholipase A1 precursor, *Dolichovespula maculata* phospholipase A1 2, *Dolichovespula maculata* phospholipase A1 1 and *Vespa maculifrons* phospholipase A1, respectively.

The product of the asymmetric PCR was cloned using pCR®4-TOPO® vector. Screening of the resulting clones led to the isolation of four clones (Viz. TMS2.1C3, TMS2.2C5, TMS2.2C10 and TMS2.2C14). On sequencing these clones encode for different isoforms of *Polistes dominulus* venom Phospholipase A1. Clones TMS2.1C3, TMS2.2C10 and TMS2.2C14 were given the names *Polistes dominulus* venom phospholipase A1 2 (allergen Pol d 1.02), *Polistes dominulus* venom phospholipase A1 3 (allergen Pol d 1.03) and *Polistes dominulus* venom phospholipase A1 4 (allergen Pol d 1.04), respectively. The nucleotide sequences of these clones are not shown but they are accessible from the Genbank Accession numbers: AY566646, AY566647 and AY566649. Their mature enzymes have calculated molecular weights of 33516.4, 33476.3 and 33480.3 Dalton, respectively. The mature enzyme of clone

TMS2.2C5 has a calculated molecular weight of 33430.3 Dalton. The nucleotide sequence of this clone is shown in Figure 5. These calculated molecular weights are comparable with those of the other members of the family: Vespidae. Phospholipases A1 of *Dolichovespula maculata* (Dol m 1 & Dol m 1.02), *Vespula maculifrons*, *Polistes annularis* and *Polistes gallicus* have molecular weights of 33745, 33780, 33539, 33483.5† and 33475 Dalton, respectively [22, 11, †: calculated by the authors based on its unpublished amino acid sequence which is accessible from Genbank Accession Number: AF174527, 20].

The amino acid polymorphism among the *Polistes dominulus* phospholipase A1 isoforms is shown in Table 1. Similar situations have been mentioned in the pub-

Pol_d_1.04	-----ADDITTLRNGTLDRGITPDCTFNEKDIELHVVYERDRNG
Pol_d_1.03	-----ADDITTLRNGTLDRGITPDCTFNEKDIELHVVYERDRNG
Pol_d_1.01	MNFKYSILFICFVKVLDNCAADDITTLRNGTLDRGITPDCTFNEKDIELHVVYERDRNG
Pol_d_1.02	-----ADDITTLRNGTLDRGITPDCTFNEKDIELHVVYERDRNG
TMS2.2C5	-----GITPDCTFNEKDIELHVVYERDRNG
PA1_POLAN	-----MSPDCTFNEKDIELHVVYERDRNG
PA1_VESVU	-MEENMNLKYLILFVYFVQVINCYGHGDPISYELDROGPKCPNSDPTVSIITETRENHR
PA1_VESMC	-----GPKCPNSDPTVSIITETRENHR
PA11_DOLMA	-----RLIMPVG-----DPSSSNELDRFSVCPPSHOTPKRIPLTRENKHK
PA12_DOLMA	-----GILPEKLVPEEISFVLSTRENKHK
Pol_d_1.04	
Pol_d_1.03	
Pol_d_1.01	
Pol_d_1.02	
TMS2.2C5	
PA1_POLAN	
PA1_VESVU	
PA1_VESMC	
PA11_DOLMA	
PA12_DOLMA	
Pol_d_1.04	IILKKEILKNYDLFQKSSQIS-HQIATLTHGFLSTGNNENFDAMAKALIETDNFLVTSVDW
Pol_d_1.03	IILKKEILKNYDLFQKSSQIS-HQIATLTHGFLSTGNNENFDAMAKALIETDNFLVTSVDW
Pol_d_1.01	IILKKEILKNYDLFQKSSQIS-HQIATLTHGFLSTGNNENFDAMAKALIETDNFLVTSVDW
Pol_d_1.02	IILKKEILKNYDLFQKSSQIS-HQIATLTHGFLSTGNNENFDAMAKALIETDNFLVTSVDW
TMS2.2C5	IILKKEILKNYDLFQKSSQIS-HQIATLTHGFLSTGNNENFDAMAKALIETDNFLVTSVDW
PA1_POLAN	IILKKEILKNYDLFQKSSQIS-HQIATLTHGFLSTGNNENFDAMAKALIETDNFLVTSVDW
PA1_VESVU	DLTYTLQTLQNHPEFKKKTIT-EQVVELTHGFLSTGNNENFDAMAKALIETDNFLVTSVDW
PA1_VESMC	DLTYTLQTLQNHPEFKKKTIT-EQVVELTHGFLSTGNNENFDAMAKALIETDNFLVTSVDW
PA11_DOLMA	DLTYTLQTLQNHPEFKKKTIT-EQVVELTHGFLSTGNNENFDAMAKALIETDNFLVTSVDW
PA12_DOLMA	DLTYTLQTLQNHPEFKKKTIT-EQVVELTHGFLSTGNNENFDAMAKALIETDNFLVTSVDW
Pol_d_1.04	
Pol_d_1.03	
Pol_d_1.01	
Pol_d_1.02	
TMS2.2C5	
PA1_POLAN	
PA1_VESVU	
PA1_VESMC	
PA11_DOLMA	
PA12_DOLMA	
Pol_d_1.04	KKGACNAFASTNDVLGYSQAVGNTRHVGYVADITKLLVEQYKVPMSNIRLIGHSLGANT
Pol_d_1.03	KKGACNAFASTNDVLGYSQAVGNTRHVGYVADITKLLVEQYKVPMSNIRLIGHSLGANT
Pol_d_1.01	KKGACNAFASTNDVLGYSQAVGNTRHVGYVADITKLLVEQYKVPMSNIRLIGHSLGANT
Pol_d_1.02	KKGACNAFASTNDVLGYSQAVGNTRHVGYVADITKLLVEQYKVPMSNIRLIGHSLGANT
TMS2.2C5	KKGACNAFASTNDVLGYSQAVGNTRHVGYVADITKLLVEQYKVPMSNIRLIGHSLGANT
PA1_POLAN	KKGACNAFASTNDVLGYSQAVGNTRHVGYVADITKLLVEQYKVPMSNIRLIGHSLGANT
PA1_VESVU	QTAACNEAAGLKLYYPPTAARNTRLVGYVADITKLLVEQYKVPMSNIRLIGHSLGANT
PA1_VESMC	QTAACNEAAGLKLYYPPTAARNTRLVGYVADITKLLVEQYKVPMSNIRLIGHSLGANT
PA11_DOLMA	QTAACNEAAGLKLYYPPTAARNTRLVGYVADITKLLVEQYKVPMSNIRLIGHSLGANT
PA12_DOLMA	QTAACNEAAGLKLYYPPTAARNTRLVGYVADITKLLVEQYKVPMSNIRLIGHSLGANT
Pol_d_1.04	SGFAGKEVQRLKLGKYKEIIGLDPAGPSFLTNKCPDRICETDAEYVQAHTSAILGVYYN
Pol_d_1.03	SGFAGKEVQRLKLGKYKEIIGLDPAGPSFLTNKCPDRICETDAEYVQAHTSAILGVYYN
Pol_d_1.01	SGFAGKEVQRLKLGKYKEIIGLDPAGPSFLTNKCPDRICETDAEYVQAHTSAILGVYYN
Pol_d_1.02	SGFAGKEVQRLKLGKYKEIIGLDPAGPSFLTNKCPDRICETDAEYVQAHTSAILGVYYN
TMS2.2C5	SGFAGKEVQRLKLGKYKEIIGLDPAGPSFLTNKCPDRICETDAEYVQAHTSAILGVYYN
PA1_POLAN	SGFAGKEVQRLKLGKYKEIIGLDPAGPSFLTNKCPDRICETDAEYVQAHTSAILGVYYN
PA1_VESVU	SGFAGKEVQRLKLGKYKEIIGLDPAGPSFLTNKCPDRICETDAEYVQAHTSAILGVYYN
PA1_VESMC	SGFAGKEVQRLKLGKYKEIIGLDPAGPSFLTNKCPDRICETDAEYVQAHTSAILGVYYN
PA11_DOLMA	SGFAGKEVQRLKLGKYKEIIGLDPAGPSFLTNKCPDRICETDAEYVQAHTSAILGVYYN
PA12_DOLMA	SGFAGKEVQRLKLGKYKEIIGLDPAGPSFLTNKCPDRICETDAEYVQAHTSAILGVYYN

Fig. 6. The global alignment

Abbreviations: Pol_d_1.04: *Polistes dominulus* venom phospholipase A1; PA1: *Polistes annularis* phospholipase A1 precursor; PA12_DOLMA: *Dolichovespula maculata* phospholipase A1 precursor.

Consensus key: * = similarity of weak groups; - = no

published literatures. For *maculata* venom phospholipase A1 2 (Dol m 1.02) and tyrosine for phenylalanine. In *Polistes* wasp sting patients [20]. Also, IgE sensitive patients using venom and nucleotide sequence for allergen. Also, it is ne-

Table 1
The amino acid polymorphisms among the different isoforms of *Polistes dominulus* venom phospholipase A1

	aa. no. 12	aa. no. 119	aa. no. 176	aa. no. 180	aa. no. 182	aa. no. 259	aa. no. 289
Partial amino acid sequence	D	T	S	X	X	S	E
Pol d 1.01	D	T	N	N	L	P	D
	GAT	ACA	AAT	AAC	CTT	CCA	GAT
TMS2.2C5	G	I	S	D	L	P	D
	GGT	ATA	AGT	GAT	TTG	CCA	GAT
Pol d 1.02	D	T	N	N	L	P	E
	GAT	ACA	AAT	AAC	CTT	CCA	GAA
Pol d 1.03	D	T	S	D	L	P	D
	GAT	ACA	AGT	GAT	TTG	CCA	GAT
Pol d 1.04	D	T	S	D	L	S	E
	GAT	ACA	AGT	GAT	TTG	TCA	GAA
One nucleotide difference	One nucleotide difference	One nucleotide difference	One nucleotide difference	Two nucleotides difference	Two nucleotides difference	One nucleotide difference	One nucleotide difference

Amino acids were numbered according to their positions in the *Polistes dominulus* phospholipase native protein (i.e. leading peptide and pro-peptide are excluded).

Abbreviations: aa. no.: amino acid number, Pol d 1.01: *Polistes dominulus* venom phospholipase A 1 1 (Allergen Pol d 1.01) (Precursor); Pol d 1.02: *Polistes dominulus* venom phospholipase A 1 2 (Allergen Pol d 1.02); Pol d 1.03: *Polistes dominulus* venom phospholipase A 1 3 (Allergen Pol d 1.03); Pol d 1.04: *Polistes dominulus* venom phospholipase A 1 4 (Allergen Pol d 1.04)

Amino acid sequence of wasp

such molecule. Natural attempts to modulate the specific IgE production and, in addition, characterization and control over allergen start *in vivo* and immunologic

1. Andrews, D. W., Girardi, N. proteins and peptides. *Mill* 345.
2. Blanca, M., Garcia, F., Mira, J., Juarez, C. (1991) Determination of *Vespa crabro* in sera of patients. *Ann. Allergy* 66: 1-5.
3. Borriello, F., Krauter, K. S. The family is delineated using a DNA probe. *J. Allergy Clin. Immunol.* 54: 5481-5487.
4. Fernandez, J., Rodas, F., M. case report. *Allergol. Immunol.* 1991; 1: 1-5.
5. Fitch, C. D., Hoffman, D. R. The allergen. *J. Allergy Clin. Immunol.* 1991; 1: 1-5.
6. Guralnick, M. W., Benton, J. American Academy of Allergy and Immunology. *Milwaukee* 1991; 1: 1-5.
7. Habermann, E., Hardt, K. I. phospholipases. *Anal. Biochem.* 1991; 1: 1-5.
8. Hoffman, D. R. (2003) *Hymenoptera Allergy*. Levine, M. and Immunology. Milwaukee 2003; 1: 1-5.
9. Hoffman, D. R. (2003) *Venoms*. Accession No. P81656.
10. Hoffman, D. R., Severino, M. The allergen in European *Polistes* wasps. *J. Allergy Clin. Immunol.* 1991; 1: 1-5.
11. Hoffman, D. R. (1994) Allergens of two vespid venom phospholipases. *J. Allergy Clin. Immunol.* 1994; 1: 1-5.
12. King, T. P., Sobolka, A. K., et al. of white-face hornet, yellow hornet. *Arch. Allergy Immunol.* 1991; 1: 1-5.
13. King, T. P., Spangfort, M. *Arch. Allergy Immunol.* 1991; 1: 1-5.
14. Landolt, P. J., Reed, H. G. (HYMENOPTERA: VESPIDAE). *J. Allergy Clin. Immunol.* 1991; 1: 1-5.
15. Levine, M. I., Lockey, R. F. Allergy, Asthma and Immunology. *Milwaukee* 1991; 1: 1-5.
16. Lichtenstein, L. M., Valent, J. *J. Allergy Clin. Immunol.* 1991; 1: 1-5.
17. Loveless, M. H., Fackeler, W. *J. Allergy Clin. Immunol.* 1991; 1: 1-5.
18. McNairy, M. M., Gastmeyer, J. *J. Allergy Clin. Immunol.* 1991; 1: 1-5.

GAA	TCA	TTG	GAT	ACA	GAT
One nucleotide difference	One nucleotide difference	Two nucleotides difference	Two nucleotides difference	One nucleotide difference	One nucleotide difference
Amino acids were numbered according to their positions in the <i>Polistes dominulus</i> phospholipase native protein (i.e. leading peptide and pro-peptide are excluded).					
Abbreviations: aa. no.: amino acid number, Pol d 1.01: <i>Polistes dominulus</i> venom phospholipase A 1 1 (Allergen Pol d 1.01) (Precursor); Pol d 1.02: <i>Polistes dominulus</i> venom phospholipase A 1 2 (Allergen Pol d 1.02); Pol d 1.03: <i>Polistes dominulus</i> venom phospholipase A 1 3 (Allergen Pol d 1.03); Pol d 1.04: <i>Polistes dominulus</i> venom phospholipase A 1 4 (Allergen Pol d 1.04)					

such molecule. Natural and synthetic forms of these determinants can then be used to attempt to modulate the specific immune response to each allergen by reducing specific IgE production and increasing specific IgG blocking antibody production. In addition, characterization of proteins will eventually allow a much higher degree of control over allergen standardization and quality than is now possible with the use of *in vivo* and immunologic techniques.

REFERENCES

- Andrews, D. W., Girardi, M., Mark, J. (1990) Chemistry and sequencing of covalently immobilized proteins and peptides. Millipore Corporation, Marlborough, Massachusetts, technical literature MG 345.
- Blanca, M., Garcia, F., Miranda, A., Carmona, M. J., Garcia, J. Fernandez, J. Terrados, S., Vega, J. M., Juarez, C. (1991) Determination of IgE antibodies to *Polistes dominulus*, *Vespa germanica* and *Vespa crabro* in sera of patients allergic to vespids. *Allergy* 46, 109–114.
- Borriello, F., Krauter, K. S. (1990) Reactive site polymorphism in the murine protease inhibitor gene family is delineated using a modification of the PCR reaction (PCR + 1). *Nucleic Acids Research* 18, 5481–5487.
- Fernandez, J., Rodes, F., Marti, J., Blanca, M. (1992) Wasp sting anaphylaxis as a cause of death: a case report. *Allergol. Immunopathol. (Madr)*. 20, 40–41.
- Fitch, C. D., Hoffman, D. R., Schmidt, M. (2001) Cloning of a paper wasp venom serine protease allergen. *J. Allergy Clin. Immunol.* 107, S221.
- Guralnick, M. W., Benton, A. W. (1995) *Entomological Aspects of Insect Sting Allergy*. Milwaukee, American Academy of Allergy and Immunology, pp. 7–20.
- Habermann, E., Hardt, K. L. (1972) A sensitive and specific plate test for the quantitation of phospholipases. *Anal. Biochem.* 50, 163–173.
- Hoffman, D. R. (2003) Hymenoptera venoms: Composition, standardization, stability. In: *Monograph on Insect Allergy*. Levine, M. I., Lockey, R. F. (eds) 4th edition, American Academy of Allergy, Asthma and Immunology, Milwaukee WI, pp. 37–53.
- Hoffman, D. R. (2003) Venom allergen 5 (Antigen 5) (Ag5) (Allergen Pol d 5). Published in DataBase only. Accession No. P81656.
- Hoffman, D. R., Severino, M. G., Campi, P., Turillazzi, S., Zerboni, R. (1998) Protease is an important allergen in European *Polistes* wasp venom allergy. *J. Allergy Clin. Immunol.* 101, S33.
- Hoffman, D. R. (1994) Allergens in hymenoptera venom. XXVI: The complete amino acid sequences of two vespid venom phospholipases. *Int. Arch. Allergy Immunol.* 104, 184–190.
- King, T. P., Sobotka, A. K., Alagon, A., Kochoumian, L., Lichtenstein, L. M. (1978) Protein allergens of white-face hornet, yellow hornet and yellow jacket venoms. *Biochemistry* 17, 5165–5174.
- King, T. P., Spangfort, M. D. (2000) Structure and biology of stinging insect venom allergens. *Int. Arch. Allergy Immunol.* 123, 99–106.
- Landoli, P. J., Reed, H. C., Aldrich, J. R., Antonelli, A. L., Dickey, C. (1999) Social wasps (HYMENOPTERA: VESPIDAE) trapped with acetic acid and isobutanol. *Florida Entomologist* 82, 609–614.
- Levine, M. I., Lockey, R. F. (2003) *Monograph on Insect Allergy*. 4th edition, American Academy of Allergy, Asthma and Immunology, Milwaukee WI.
- Lichtenstein, L. M., Valentine, M. D., Sobotka, A. K. (1979) Insect allergy: The state of the art. *J. Allergy Clin. Immunol.* 64, 5–12.
- Loveless, M. H., Fackeler, W. R. (1959) Wasp venom allergy and immunity. *Ann. Allergy* 14, 347–366.
- McNairy, M. M., Gastmeyer, J., Pantera, B., Hoffman, D. R. (2000) Isolation of paper wasp venom protease by affinity chromatography. *J. Allergy Clin. Immunol.* 105, S57.

19. Nielsen, H., Engelbrecht, J., Brunak, S., von Heijne, G. (1997) Identification of prokaryotic and eukaryotic signal peptides and prediction of their cleavage sites. *Protein Engineering* 10, 1–6.
20. Pantera, B., Hoffman, D. R., Carresi, L., Cappugi, G., Turillazzi, S., Manau, G., Severino, M., Spadolini, I., Orsomando, G., Moneti, G., Pazzagli, L. (2003) Characterization of the major allergens purified from the venom of the paper wasp *Polistes gallicus*. *Biochim. Biophys. Acta* 1623, 72–81.
21. Panzani, R., Blanca, M., Sanchez, F., Juarez, C. (1994) Sensitivity to European wasps in a group of allergic patients in Marseille: preliminary results. *J. Investig. Allergol. Clin. Immunol.* 4, 42–46.
22. Soldatova, L., Kochoumian, L., King, T. P. (1993) Sequence similarity of a hornet (*D. maculata*) venom allergen phospholipase A₁ with mammalian lipases. *FEBS Letters*, 320, 145–149.
23. Wahle, E., Kuhn, U. (1997) The mechanism of 3' cleavage and polyadenylation of eukaryotic pre-mRNA. *Prog. Nucleic Acid Res. Mol. Biol.* 57, 41–70.
24. Zhao, J., Hyman, L., Moore, C. (1999) Formation of mRNA 3' ends in eukaryotes: Mechanism, regulation, and interrelationships with other steps in mRNA synthesis. *MMBR* 63, 405–445.

INFLUENCE ON THE LIVER W

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Acetaminophen (AAP), acetylsalicylic acid (ASA) are drugs that have wide use in health care. In this work, technetium-99m (^{99m}Tc). This was used to label blood elements with ^{99m}Tc. Blood elements were labeled with ^{99m}Tc-labeled process. Plasma and blood fractions were separated and analyzed. The results have shown that the antipyretic effect of ASA on the blood elements when the blood is taken from beings. Although the experimental results show that DIP should not interfere with the results with ^{99m}Tc.

Keywords: Acetaminophen – acetylsalicylic acid

Acetaminophen, acetylsalicylic acid (ASA) are drugs that have wide use and share in common. The antipyretic and analgesic effect of ASA is due to cyclooxygenase that is an enzyme that produces some autacoids [6, 9, 10], which is responsible for the inflammatory action by the release of prostaglandins [2, 3, 39], while acetaminophen does not. However, acetylsalicylic acid has been used at therapeutics doses as: (i) for the treatment of bone pain [26, 29], (ii) for the treatment of hemorrhage and exacerbation of bleeding [18] and (iv) in the treatment of

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