Aromatic Amino Acids in the Venom of the Braconid Parasitoid *Apanteles kariyai*

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The venom gland of the braconid parasitoid, *Apanteles kariyai,* was morphologically observed with photomicroscope and aromatic amino acids in its venom fluid were chemically analyzed with a three-dimensional HPLC system-coulometric ECD. Tyrosine (TYR-4) → tyramine (TYRA) → hydroxyphenylacetic acid (HPAC-4) and tryptophan (TRP) → kynurenine (KYN) were the detected metabolic pathways. This report of venom components of a braconid wasp outlines qualitative differences between this and venom from social wasps.

The function of the venom of most endoparasitoids has not been well-documented in contrast to that of the paralyzing venoms of ectoparasitoids [1, 2]. The venom fluids of some Braconidae have been reported to synergize the effects of the polydnaviruses (poly-DNA-viruses) by affecting the physiological environment of their host [3], and promoting uncoating and persistence of viral DNA [4]. Venom components, therefore, appear very important in successful parasitism, but venom composition is not clear, especially with regard to the precise nature and quantity of biogenic amines and related metabolites.

Abbreviations: TYR-4, tyrosine; NMN, normetanephrine; KYN, kynurenine; VA, vanillic acid; TYRA, tyramine; TRP, tryptophan; HPAC-4, hydroxyphenylacetic acid; HVA, homovanillic acid.

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Materials and Methods

Insects

Larvae of the common armyworm, *Leucania separata,* were reared on an artificial diet (Yakult Co., Japan) at 25 ± 1 °C under a 16:8 LD photoperiod. Adult *Apanteles kariyai,* a gregarious parasite, were maintained in glass tubes with a cotton pad soaked with 30% honey solution.

Dissection of venom glands

The venom apparatus of *A. kariyai* females lies dorsal to the ovaries and, as shown in Fig. 1, it consists of a venom gland (VG) and two venom filaments (VF) (showed only one in Fig. 1). The VFs are approximately 55 µm wide and 720 µm in length. The diameter of the VG is approximately 250 µm. The venom filaments and venom glands (20 pairs of glands) were individually excised from

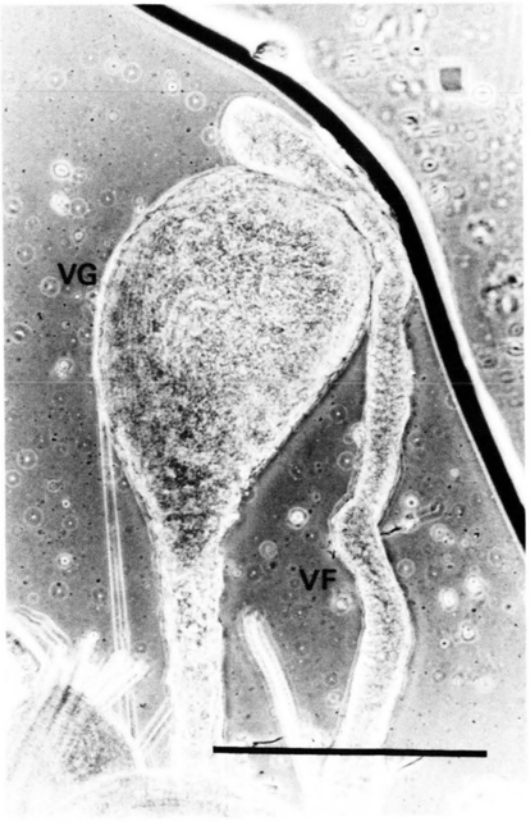

Fig. 1. Photograph of the venom gland and venom filament. VG: venom gland, VF: venom filament, Scale bar: 250 µm.

20 mated *A. kariyai* females and extensively rinsed with 0.9% NaCl solution. They were collected by grasping the ducts leading to the ovipositor with forceps and removing them. Once excised, the glands were placed in 740 µl of ice cold 0.9% NaCl solution in 1 ml microfuge tubes. After vigorous vortexing, samples were centrifuged (363 × *g*, 5 min) to remove tissue fragments. The supernatant was analyzed using an HPLC-electrochemical detector (ECD) (Coulochem Electrode Array System, Neurochem ESA, Bedford, M.A.).

Preparation of HPLC sample

The supernatant (740 µl) containing venom was mixed with 64 µl 60% perchloric acid (PCA) solution and then centrifuged at 3,636 × *g* for 10 min to precipitate the remaining protein. The supernatant was then filtered on a Centricon type filter (Ultrafree-C3, Millipore) at 10,000 rpm for 10 min. The analytical method and equipment were identical to those described by Shimizu *et al.* [5] or Takeda *et al.* [6]. We used standards of analytical reagent grade to identify unknowns.

Results and Discussion

Table I shows the levels of biogenic amines and their precursors in the venom fluid. Tyrosine (TYR-4), tryptophan (TRP), tyramine (TYRA), kynurenine (KYN), hydroxyphenylacetic acid (HPAC-4), vanillic acid (VA), homovanillic acid (HVA), and normetanephrine (NMN) as aromatic amino acids were detected. TYR-4 → TYRA → HPAC-4 and TRP → KYN was the only detected metabolic pathway in the venom fluid of *A. kariyai*.

In most hymenopterans (social bees and wasps) the venom is used as a defense against other arthropods and mammalian predators.

Table I. Levels of biogenic amines and their precursors in the soluble components of the venom glands and venom filaments of the braconid parasitoid *A. kariyai*.

Compound	pg/Venom gland + filaments	Compound	pg/Venom gland + filaments
TYR-4	647 ± 100	TYRA	6 ± 1
NMN	14 ± 13	TRP	57 ± 11
KYN	92 ± 63	HPAC-4	12 ± 11
VA	41 ± 61	HVA	1 ± 1

Three separate samples were analyzed and each sample was prepared from 20 pairs of venom filaments and glands. Mean ± SD.

Biogenic amines in the venom of social wasps have been characterized extensively [see 7]. In the venom of social wasps, dopamine, noradrenaline, adrenalin and serotonin appear to be the primary components [7]. Venom components serve as paralyzing agents and poisons injected into prey or for defensive roles against natural enemies.

On the other hand, the function of the venoms of the braconid parasitoids seems to be to enhance the virus, to prevent host encapsulation or to act on host hemocytes to "protect" the parasite [see 8]. In the present experiments, biogenic amines characteristic of the venom of social wasps were not found. Thus, there are major qualitative differences in the detected biogenic amines of social versus parasitic wasps.

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[1] R. L. Beard, Venoms of Braconidae, in: Handbuch der Experimentellen Pharmakologie (S. Bettini, ed.), **Vol. 48,** pp. 773–800, Springer Verlag, Berlin 1978.
[2] T. Piek, in: Venoms of the Hymenoptera: Biochemical, Pharmacological and Behavioral Aspects (T. Piek, ed.), Academic Press, New York 1986.
[3] T. Tanaka and S. B. Vinson, Ann. Ent. Soc. **84,** 87–92 (1991).
[4] D. B. Stoltz, D. Guzo, E. R. Belland, C. J. Lucarotti, and E. A. Mackinnon, J. Gen. Virol. **69,** 903–907 (1988).
[5] T. Shimizu, M. Mihara, and N. Takeda, J. Chromatogr. **539,** 193–197 (1991).
[6] N. Takeda, H. Takaoka, T. Shimizu, M. Yazawa, and S. Yagi, Comp. Biochem. Physiol. **100C,** 677–682 (1991).
[7] J. Ishay, Z. Abraham, Y. Grunfeld, and S. Gitter, Comp. Biochem. Physiol. **48A,** 369–373 (1974).
[8] N. E. Beckage, Parasitic Effects on Host Development, in: New Directions in Biological Control (R. R. Baker and P. E. Dunn, eds.), pp. 497–515, A. R. Liss, New York 1990.