Makisterone A: its distribution and physiological role as the molting hormone of true bugs[1]

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Summary. Makisterone A, a 28-carbon (C-24 alkyl) hexahydroxy steroid, has been identified by mass spectrometry as the major ecdysteroid in last-stage larvae of the large milkweed bug, *Oncopeltus fasciatus*, a phytophagous hemipteran. Similarly, it is a major molting hormone in 2 phytophagous and 1 predacious species of Hemiptera belonging to the group, Pentatomomorpha. It is not, however, a major ecdysteroid in another group of Hemiptera, the Cimicomorpha, where 1 predacious and 2 hematophagous species contain ecdysone and 20-hydroxyecdysone as their major molting hormones.
Key words. Milkweed bug; *Oncopeltus fasciatus;* makisterone A; molting hormone; ecdysteroid.

Except makisterone A, all known naturally occurring ecdysteroids of insects are C_{27} steroids[2]. This C_{28} steroid, 2β, 3β, 14α, 20, 22, 25-hexahydroxy-24ξ-methyl-5β-cholest-7-ene-6-one (**1**), has been identified by nuclear magnetic resonance and mass spectrometry as the major ecdysteroid in the developing egg of the large milkweed bug, *Oncopeltus fasciatus*[3], and by high pressure liquid chromatography (HPLC) and radioimmunoassay (RIA), as the major ecdysteroid in the hemolymph of last-stage *Oncopeltus* larvae[4] and in 3 other phytophagous and 1 predacious hemipteran[5,6] including mass spectral identification in *Dysdercus fasciatus*[6]. This identification of makisterone A as the major ecdysteroid in some larval Hemiptera stands in sharp contrast to the situation in other larval insects and to the accepted role of 20-hydroxyecdysone (**2**), a C_{27} steroid, and its prohormone, ecdysone (**3**), as the active molting hormones of arthropods[7]. Makisterone A has been shown to be more active than 20-hydroxyecdysone in inducing cuticulogenesis in larval and adult *Oncopeltus*[4,8] and in affecting development of *Oncopeltus* embryos[9]. We now positively identify makisterone A by mass spectrometry as the major molting hormone of last-stage *Oncopeltus* larvae and examine its distribution in 3 species of hematophagous and predacious Hemiptera.

Materials and methods. The techniques for extraction of ecdysteroids, RIA and HPLC have been reported previously[4,5]. Ecdysteroids were separated on a reverse-phase C18 μBondapak column (Waters Associates, Milford, MA; 3.9 mm i.d. × 37 cm, 10 μm particle size) at 2 ml/min with 40% methanol. The retention times of unknown ecdysteroids were compared to the retention times of authentic standards obtained from Sigma and further purified by HPLC. Radioimmunoassay procedures and antibody ecdysteroid affinities were reported previously[10,11]. The ratio of the mass of 20-hydroxyecdysone or makisterone A required to displace 50% of the labeled ecdysone ([23,24-^{3}H(N)]-ecdysone, 60–80 Ci/mmole, New England Nuclear) relative to the mass of ecdysone required is 2.8. Following purification by HPLC, ecdysteroids were prepared for mass spectrometry by trimethylsilane derivatization[12]. Mass spectra were obtained with a Hewlett Packard 5995 GC-MS utilizing electron impact at 70 eV. A 45 cm × 3 mm glass column packed with 1% OV 101 on Gas Chrom A (80–100 mesh) was operated at 290 °C with helium flow of 30 ml/min.

For each species examined the ecdysteroid titer of the hemolymph was determined throughout the last larval stage by RIA, and the major ecdysteroids present during the time of peak titer (i.e. at approximately the time of apolysis) were determined by HPLC/RIA.

Results and discussion. Mass spectral analysis of the major ecdysteroid of last-stage *Oncopeltus* larvae, gave a mass spectrum identical to an authentic standard of makisterone A with essential peaks at m/e 561, characteristic of the steroid nucleus, and m/e 185, characteristic of a side chain containing an extra CH_3 group at C-24. Retention times for both samples were 1.8 min.

Makisterone A is present as the major ecdysteroid in *Oncopeltus* last-stage larvae, representing 68.5% of the total hemolymph ecdysteroids (table). It is also one of the major ecdysteroids in the phytophagous southern green stink bug, *Nezara viridula*, and the red cotton stainer bug, *Dysdercus cingulatus*. Even the closely related pentatomid, the spined soldier bug, *Podisus maculiventris*, a secondarily predacious species, produces makisterone A when fed on mealworm pupae. In these species, makisterone A represents from 19.3 to 68.5% of the total ecdysteroid in the hemolymph of last-instar larvae, whereas ecdysone and 20-hydroxyecdysone represent only 0–4.7%. The level of these latter ecdysteroids is near the limit of detection by HPLC/RIA and therefore, their absolute pres-

Major ecdysteroids of larval Hemiptera[1]

Family	Species	Major ecdysteroids HPP	20-HE	MAK-A	ECD	LPP	Feeding preference
		Pentatomomorpha[24]					
Pentatomidae	*Nezara viridula*	5.3 (44)	2.7 (22)	36.1 (297)	1.2 (10)	40.6 (333)	Green beans
	Podisus maculiventris	7.1 (104)	3.5 (51)	19.3 (282)	1.3 (19)	58.0 (847)	Mealworm pupae
Lygaeidae	*Oncopeltus fasciatus*	6.1 (81)	4.7 (63)	68.5 (911)	1.0 (13)	4.9 (65)	Milkweed seeds
Pyrrhocoridae	*Dysdercus cingulatus*	38.9 (224)	0 (0)	41.5 (239)	0 (0)	2.4 (14)	Cotton seeds
		Cimicomorpha[24]					
Reduviidae	*Rhodnius prolixus*	5.1 (338)	54.7 (3624)	1.6 (106)	38.9 (2577)	0 (0)	Blood
	Arilus cristatus	7.2 (28)	20.8 (82)	1.0 (40)	66.9 (264)	0 (0)	Mealworm pupae
Cimicidae	*Cimex lectularius*	27.3 (571)	39.5 (826)	3.6 (75)	8.4 (176)	0 (0)	Blood

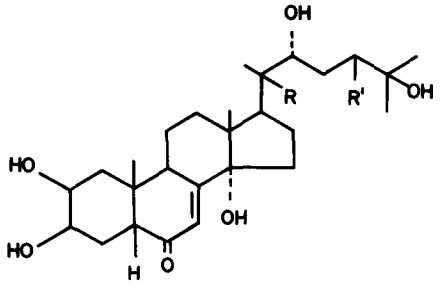
[1] Relative percentages of total ecdysteroids determined from the RIA activity of HPLC fractions (numbers in parentheses are expressed as picograms of 20-HE equivalents per μl of hemolymph). The remainder of the ecdysteroids for those that total less than 100% were unidentified minor components except in the case of *C. lectularius* where a peak containing 19.3% of the total activity was found between HPP (highly polar peak) and 20-HE (20-hydroxyecdysone). Other abbreviations are makisterone A (MAK-A), ecdysone (ECD) and less polar peak (LPP), the latter eluting between ecdysone and 2-deoxyecdysone. 3-Epi-20-hydroxyecdysone is not effectively resolved from 20-HE by our separation system and therefore may be reflected in the percentages of 20-hydroxyecdysone. Retention times in minutes for these ecdysteroids were HPP (2.4), 20-HE (5.5), MAK-A (7.6), ECD (10.7) and LPP (15.7). Relative percentages have not been corrected for antibody affinities since these are not known for the HPP and LPP. No sexual differences were observed. Relative percentages of the Pentatomomorpha were derived from previously reported data[4,5] and included for comparison.

ence is questionable. Although there are other major ecdysteroids in 3 of the 4 species, their chemical structures and physiological roles have yet to be elucidated. It is clear, however, that in *Oncopeltus*[4,8] and *Dysdercus*[13] purified preparations of makisterone A are more active in inducing cuticle production than ecdysone or 20-hydroxyecdysone. Furthermore, *Pyrrhocoris apterus* larvae are very insensitive to 20-hydroxyecdysone injections[14]. With respect to the quantitative distribution of these ecdysteroids in the Pentatomomorpha and the insensitivity of species within this group to ecdysone or 20-hydroxyecdysone, other ecdysteroids, one of which is makisterone A, must act as the molting hormones in the Hemiptera-Pentatomomorpha.

A similar determination of the major ecdysteroids in the Cimicomorpha revealed a different distribution of ecdysteroids in these species. In one of the hematophagous species examined, *Rhodnius prolixus,* ecdysone and 20-hydroxyecdysone were the major ecdysteroids, representing 38.9 and 54.7% of the total hemolymph ecdysteroids (table). Our results for *Rhodnius* corroborate the report by Steel et al.[15] that ecdysone and 20-hydroxyecdysone are the major hemolymph ecdysteroids in last-stage larvae of this species. In another hematophagous species, the bed bug, *Cimex lectularius,* 20-hydroxyecdysone was the major ecdysteroid. In contrast to the situation in the pentatomid, *Podisus maculiventris,* a member of the Pentatomomorpha, when the predacious wheel bug, *Arilus cristatus,* was fed on mealworm pupae, it produced ecdysone and 20-hydroxyecdysone as the major ecdysteroids, representing 66.9 and 20.8% of the total hemolymph ecdysteroids, respectively. In these 3 species of the Cimicomorpha, makisterone A represented only 1.0 to 3.6% of the total ecdysteroids, again approaching the limit of detectability by HPLC/RIA. Larvae of *Rhodnius* are highly sensitive to 20-hydroxyecdysone stimulation of molting[16], in contrast to the situation in the Pentatomomorpha.

Ecdysteroids are synthesized by insects from cholesterol which in hematophagous and predacious insects is obtained from their host. Phytophagous insects must convert C_{28} and C_{29} phytosterols, mainly campesterol, sitosterol, and stigmasterol (C-24 alkyl sterols), by dealkylation primarily to cholesterol[17–20]. However, of 3 species of the Pentatomomorpha so far examined, none were capable of dealkylating phytosterols[6,21,22]. As previously suggested by Kaplanis et al. for *Oncopeltus*[3], these insects may be able to directly utilize C_{28} and C_{29} plant sterols without dealkylation to C_{27} sterols as precursors for their molting hormones. This mechanism, once evolved, has apparently been retained even in a secondarily predacious species of the Pentatomomorpha, *Podisus maculiventris.* When fed on mealworm pupae which contained 63.3% of their total sterols as cholesterol and 4.6% as campesterol, the most likely precursor of makisterone A[23], makisterone A was found as a major ecdysteroid in the hemolymph of last-stage larvae with little, if any, ecdysone or 20-hydroxyecdysone. Thus, even with its precursor sterol, cholesterol, readily available, ecdysone was not produced by last-stage *Podisus* larvae. On the other hand, when a species of the Cimicomorpha, *Arilus cristatus,* was fed on mealworm pupae, ecdysone and 20-hydroxyecdysone were found as the major ecdysteroids in the hemolymph. Apparently, the metabolic pathways for synthesizing ecdysteroids in insects are strongly conserved, once evolved.

Few insect species have been examined using techniques capable of distinguishing their major ecdysteroids. Our investigations demonstrate that true bugs have evolved independent mechanisms for ecdysteroid control of molting, apparently under the selective pressure of available food. Closer scrutiny of the ecdysteroid content of the phytophagous insects may reveal additional evolutionary solutions by the insects to the appearance of alkylated sterols in terrestrial plants.



1 R = OH; R' = CH_3
2 R = OH; R' = H
3 R = R' = H

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1 We thank Suzanne M. Spalding for technical assistance, W.E. Bollenbacher for providing the ecdysteroid antiserum, D.H.S. Horn for providing the 2-deoxyecdysone, and L.I. Gilbert, M.J. Loeb and A.B. DeMilo for reading this manuscript and making suggestions toward its improvement.

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