

## SAPINDACEAE, CYANOLIPIDS, AND BUGS

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**Abstract**—Scentless plant bugs (Heteroptera: Rhopalidae) are so named because adults of the Serinethinae have vestigial metathoracic scent glands. Serinethines are seed predators of Sapindales, especially Sapindaceae that produce toxic cyanolipids. In two serinethine species whose ranges extend into the southern United States, *Jadera haematoloma* and *J. sanguinolenta*, sequestration of host cyanolipids as glucosides renders these gregarious, aposematic insects unpalatable to a variety of predators. The blood glucoside profile and cyanogenesis of *Jadera* varies depending on the cyanolipid chemistry of hosts, and adults and larvae fed golden rain tree seeds (*Koelreuteria paniculata*) excrete the volatile lactone, 4-methyl-2(5H)-furanone, to which they are attracted. *Jadera* fed balloon vine seeds (*Cardiospermum* spp.) do not excrete the attractive lactone. Loss of the usual heteropteran defensive glands in serinethines may have coevolved with host specificity on toxic plants, and the orientation of *Jadera* to a volatile excretory product could be an adaptive response to save time.

**Key Words**—*Leptocoris*, *Jadera*, Heteroptera, Rhopalidae, Serinethinae, Sapindaceae, cyanogenesis, glucoside, sequestration, allomone, attractant, predation, pheromone, allelochemic.

<sup>4</sup>Mention of a commercial product does not constitute an endorsement by the USDA.

## INTRODUCTION

Rhopalidae (Heteroptera) are called scentless plant bugs because in adults of the Serinethinae the metathoracic scent gland is vestigial. However, this common name is misleading as serinethine adults retain the abdominal scent glands usually found only in immatures, and adults of the Rhopalinae actually do possess a metathoracic scent gland (Aldrich et al., 1979; Aldrich, 1988). Serinethinae prefer Sapindales, especially the Sapindaceae (Schaefer and Chopra, 1982; Schaefer and Mitchell, 1983), where they form aggregations of up to thousands of individuals (Carroll and Loye, 1987; Carroll, 1988; Wolda and Tanaka, 1987). Sapindaceae produce toxic cyanolipids in their seeds (Seigler and Kawahara, 1976); thus loss of the usual heteropteran defensive gland in serinethines may have coevolved with host specificity on these plants. This hypothesis is bolstered by the discovery that the aposematic bug, *Leptocoris isolata*, sequesters host cyanolipids as glucosides and can exude blood containing enough of these compounds to repel ants (Braekman et al., 1982).

Two serinethine species in the genus *Jadera* are common in the southern United States, *J. haematoloma* and *J. sanguinolenta*. In southern Florida, the balloon vine, *Cardiospermum corindum*, is the native host of *Jadera* (Carroll, 1988). Seeds of this plant contain cyanolipid **1** (Scheme 1) (Seigler, 1974; Seigler and Kawahara, 1976). Soapberry, *Sapindus saponaria drummondii*, the native host of *J. haematoloma* in the southwestern United States (Carroll and Loye, 1987), contains only **2** in its seeds (Seigler, 1974). The golden rain tree, *Koelreuteria paniculata*, is an introduced host of *J. haematoloma* in the southwestern and central states, and *K. elegans formosana* has likewise been introduced in Florida, where its seeds are fed upon by both *J. haematoloma* and *J. sanguinolenta* (Carroll and Loye, 1987; Carroll, 1988). *K. paniculata* seeds contain **2** and **3** (Mikolajczak et al., 1970) and *K. e. formosana* probably produces a similar cyanolipid blend (Seigler and Kawahara, 1976). We tested the acceptability of *Jadera* as prey for various predators and examined the sequestration of glycosides of cyanolipids in *Jadera* as a function of host species. Our results provide an insight into the chemical vocabulary of these semisocial bugs.

## METHODS AND MATERIALS

*Insects.* *Jadera* spp. were collected by S.P.C. in 1985, and express-mailed to J.R.A. for dissection or rearing. Cultures of *J. haematoloma* and *J. sanguinolenta* were maintained on *K. paniculata* seeds for several generations and for one generation on *C. corindum* and *C. grandiflorum* seeds, plus water.

Two lycaenids (Lepidoptera) feeding on unripe seeds of *C. corindum* were

collected by S.P.C. at Plantation Key, Florida, in February 1988 and sent to J.R.A. for analysis: *Hemiarigus thomasi* and *Clorostrymon simaethis*.

**Chemical Analysis.** Insect blood was collected in capillary tubes from amputated legs. Methanolic extracts were acetylated in pyridine-acetic anhydride (1:1), fractionated on silica gel, and monitored by TLC (silica gel 60 F<sub>254</sub> nano plates). Gas chromatography (GC) was performed using a Varian 3700 GC with a Shimadzu C-R3A recorder, on a 15-m bonded methyl silicone capillary column (0.25 mm ID; DB-1, J&W Scientific, Folsom, California) with helium as carrier (40 cm/sec) at 145°C for 2 min to 240°C at 15°/min. Urine from bugs was extracted with methylene chloride (20  $\mu$ l/100  $\mu$ l CH<sub>2</sub>Cl<sub>2</sub>), and analyzed by GC (DB-1, 45°C for 2 min to 230°C at 15°/min). The volatile excretory product and acetylated glycosides were isolated in glass capillary tubes from a DB-1 column (15 m  $\times$  0.53 mm ID) in a Varian 3700 GC equipped with a thermal conductivity detector. GC-mass spectrometry was conducted using a Finnigan 4510 GC-MS system on a 30-m DB-1 column. NMR spectra were recorded at 60 MHz on a JEOL FX-60Q FT instrument and at 300 MHz on a General Electric QE-300 instrument with TMS as an internal standard. Decoupling experiments were performed for some samples at 300 MHz using single-frequency, low-pressure, on-resonance conditions to remove the target absorption. Infrared spectra were obtained in CCl<sub>4</sub> using a Perkin-Elmer 580B spectrometer and a UV spectrum of **4** (Figure 2 below) was recorded on a Perkin-Elmer 559 UV-VIS spectrometer. 4-Methyl-2(5H)-furanone (**4**) was synthesized according to published procedures (Price and Judge, 1973; Pelletier et al., 1975).

**Cyanogenesis.** A cupric acetate-benzidine acetate test solution on filter paper strips was used to check for release of HCN from crushed bugs and urine (Feigl, 1966).  $\beta$ -Glucosidase (40  $\mu$ l, 4 mU/ $\mu$ l, 0.1 mM citrate/PO<sub>4</sub> buffer, pH 5.00; Sigma) was also added in conjunction HCN indicator tests.

**Attraction Bioassay.** Insects were anesthetized with CO<sub>2</sub> and placed in a 500-ml three-neck flask. A 250-ml splash-guard adapter was attached to each side arm, and the side arms were connected via silicone tubing to charcoal filtered and humidified air. The middle neck of the flask was connected to the house vacuum (20 ml/min), and the apparatus was positioned horizontally on a countertop in a room (23  $\pm$  2.5°C) with bright fluorescent lights. Five, 10, or 50  $\mu$ l of **4** (10  $\mu$ g/ $\mu$ l in CH<sub>2</sub>Cl<sub>2</sub>) was applied to filter paper in the upwind end of one splash-guard and the other arm had filter paper with CH<sub>2</sub>Cl<sub>2</sub> only. Treated and control sides were alternated between tests. The number of insects in each splash-guard was counted every 15 min for the first 2 hr, then hourly, and the next morning.

**Predator Aversion.** *Jadera haematoloma* from golden rain trees were offered to four toad species (*Bufo woodhousei*, *B. americanus*, *B. cognatus*,

and *B. speciosus*), the blue jay (*Cyanocitta cristata*), and praying mantids (probably Chinese mantids, *Tenodera ardifolia sinensis*).

## RESULTS

*Identification of Volatile Excretory Product.* Urine from *J. sanguinolenta* adults or larvae fed *K. paniculata* seeds had a single volatile component (Figure 1A, 4); bugs fed balloon vine seeds had no volatiles (Figure 1C). *J. haematoloma* adults and larvae also excreted 4 when fed golden rain tree seeds, but not when fed balloon vine seeds. The MS of 4 suggested a methyl-2(5H)-furanone structure. EI-MS  $m/z$  (%): 98( $M^+$ , 33), 70(5), 69(100), 68(11), 55(1), 55(3), and 50(3);  $CH_4$  CI-MS: 99( $[M+H]^+$ , 100);  $NH_3$  CI-MS: 214( $[2+NH_4]^+$ , 2), 150( $[M+(NH_3)_3H]^+$ , 10), 133( $[M+(NH_3)_2H]^+$ , 100), and 116( $[M+NH_4]^+$ , 35). The identity of 4 as 4-methyl-2(5H)-furanone (3-methyl-2-butenolide) was deduced from spectral data: UV  $\gamma_{max}$  nm: 210; IR ( $CCl_4$ ):  $\gamma C=O$  1755/1785  $cm^{-1}$ ,  $\gamma C=C$  1650  $cm^{-1}$ ;  $[^1H]NMR$  (60 MHz,  $CDCl_3$ )  $\delta$  2.16 (br s, 3H), 4.75 (br s, 2H), and 5.89 (br s, 1H); lit. (Liardon and Philipposian, 1978): IR (film):  $\gamma C=O$  1740/1775  $cm^{-1}$ ,  $\delta C=C$  1640  $cm^{-1}$ ;  $[^1H]NMR$  (60 MHz,  $CDCl_3$ )  $\delta$  2.18 (m), 4.81 (m), and 5.94 (m). Insect-derived 4 coeluted with synthetic 4 by GC.

*Identification of Blood Glycosides.* Pinching the bugs usually caused them to bleed intersegmentally and eject scent secretion. Bleeding was most prevalent at the rostrum and, in adults, from the openings of the metathoracic scent gland. Methanol extracts of exuded fluid and of hemolymph showed the same components by TLC ( $CHCl_3$ -ethanol, 60:40) indicating that the externalized fluid is blood. Acetylation of 20  $\mu l$  of blood extract from *J. sanguinolenta* larvae fed *K. paniculata* seeds confirmed by GC (Figure 1B) the pattern observed by TLC; compounds 6, 8, 12, and 14 accounted for 0.5, 2.5, 88, and 4.5% of the total peak area, respectively. GC of an acetylated extract from *J. sanguinolenta* larvae reared on balloon vine seeds exhibited a new component (10, 69%) in addition to 12 (26%), and compounds 6, 8, and 14 were absent (Figure 1D).

The major blood component from six larvae and six adults of *J. haematoloma* reared on *K. paniculata* seeds (silica gel; 3 ml fractions of 10, 20, 40, 60, and 80% methanol- $CHCl_3$ ) gave CI-MS consistent with a monoglycoside having an aglycon of mol wt 275. The presence of the nitrile group in 11 was confirmed by the IR spectrum ( $\gamma C\equiv N$  at 2228  $cm^{-1}$ ) and, since cardiospermin (=9) does not show IR absorption in the 2250  $cm^{-1}$  region (Seigler et al., 1970, 1974), this structure was ruled out. The identity of 11 was confirmed by comparison to acetylated 4- $\beta$ -D-glucopyranosyloxy-3-hydroxymethyl-2-butenyl-nitrile (=12, Figure 2) prepared from material isolated from *L. isolata*.

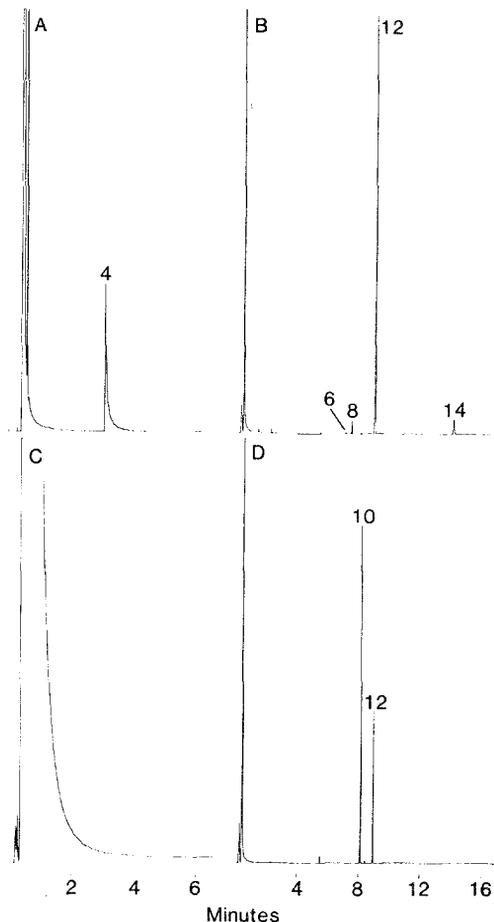


FIG. 1. Gas chromatograms of  $\text{CH}_2\text{Cl}_2$  extracts of excreta and acetylated MeOH blood extracts from *Jadera sanguinolenta* adults reared on hosts with different cyanolipids (numbering of peaks follows Figure 2): (A) fed *Koeleria paniculata*/urine; (B) fed *K. paniculata*/blood; (C) fed *Cardiospermum corindum*/urine (splitless GC injection); (D) fed *C. corindum*/blood.

For identification of minor bloodborne glycosides of *Jadera* reared on *K. paniculata* seeds, blood was collected from 166 male and female *J. sanguinolenta* in 3 ml of methanol and acetylated. Fractions (5 g silica gel) with material migrating above 11 by TLC were combined, as were those with material migrating below 11. There were two prominent compounds in the fast-eluting fractions, one with an  $\text{NH}_3$  CI-MS base peak at  $m/z$  447 corresponding to a mol wt

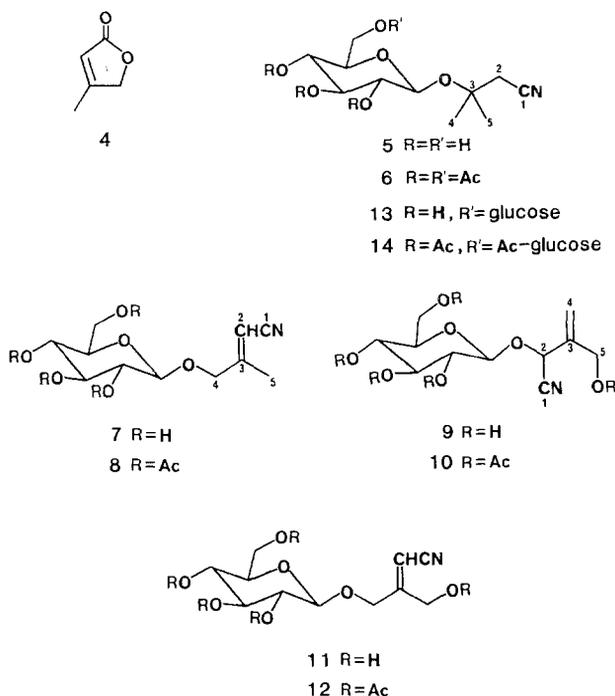


FIG. 2. Structures of *Jadera* excretory lactone and blood-sequestered glucosides.

429 (Figure 1B, **6**) and the other with a base peak at  $m/z$  445 corresponding to a mol wt 427 (Figure 1B, **8**). The  $[^1\text{H}]\text{NMR}$  of **8** differed characteristically from that of **12** by the absence of the  $\text{H}_2\text{C}-5$  signal and the upfield shift of one methyl signal. Structure **7** (Figure 2) is proposed for this minor glycoside apparently derived from cyanolipid **3**. Compound **14** was isolated from the slow-eluting material by refractionating on silica gel (1:1, 3:1, and 6:1  $\text{CHCl}_3$ -benzene) and recrystallizing from acetone-hexane (6:1  $\text{CHCl}_3$ -benzene fraction). The  $\text{NH}_3$  CI-MS of **14** exhibited a base peak at  $m/z$  735 indicating a mol wt 717. This was substantiated by the  $\text{CH}_4$  CI-MS ( $m/z$  (%) 718(20),  $[\text{M}+\text{H}]^+$ ), and ions at  $m/z$  (%) 619(35) and 331(100) suggested the presence of a dihexose moiety. Two anomeric proton doublets ( $\delta$  4.57,  $J = 7.8$  Hz;  $\delta$  4.70,  $J = 8.0$  Hz) were observed in the  $[^1\text{H}]\text{NMR}$ , indicating a  $\beta$ -linked diglycoside structure. The signal at  $\delta$  4.08–4.30 integrated to two protons ( $\text{H}_2\text{C}-6''$ ) whereas the signal at  $\delta$  3.58–3.84 integrated to four protons ( $\text{HC}-5'$ ,  $\text{HC}-5''$ , and  $\text{H}_2\text{C}-6'$ ), thus a  $\beta(1-6)$ disaccharide linkage was indicated. This interpretation was substantiated by comparisons to the  $[^1\text{H}]\text{NMR}$  spectra of amygdalin, sophorose, and melibiose. The EI-MS of **14** exhibited ions at  $m/z$  702 ( $[\text{M}-\text{CH}_3]^+$ , <1%) and

677( $[M-CH_2CN]^+$ , 1%) consistent with a 3-glycoside of 3-methylbutyronitrile (Spencer and Daxenbichler, 1980). Decoupling experiments ruled out an aglycon corresponding to saturated **7** in favor of the dimethyl aglycon. Based on the CI-MS of **6** and the similarity of the EI-MS of **6** to that of **14**, structure **5** is proposed as the monoglucoside analog of **13**.

Compound **10** was the major glycoside of an acetylated methanolic extract of blood from *J. sanguinolenta* larvae reared on seeds of *C. corindum* (Figure 1D). The  $NH_3$  CI-MS of **10** exhibited a base peak at  $m/z$  503 indicating a mol wt 485, and the EI-MS showed an  $m/z$  331 (9%) ion indicative of a hexose moiety. The IR of the isolated compound had no  $\gamma C\equiv N$  and the  $[^1H]NMR$  spectrum had a pair of singlets at 5.54  $\delta$  and 5.66  $\delta$  (vinyl, 2H), a singlet at 5.20  $\delta$  (cyanohydrin H), and was otherwise identical to the published spectrum of acetylated cardiospermin (Seigler et al., 1974).

Aliquots of blood (5–15  $\mu$ l) from the two lycaenid caterpillars collected from *C. corindum* showed no trace of the glycosides characteristic of *Jadera* feeding on balloon vine seeds.

*3-Glucopyranosyloxy-3-methylbutyronitrile (5)*. Characterized as the tetraacetate **6**.  $NH_3$  CI-MS  $m/z$  (%): 447 ( $[M+NH_4]^+$ , 100);  $^{15}NH_3$  CI-MS: 448( $[M+^{15}NH_4]^+$ , 100); EI-MS: 331( $[M-C_5H_8ON]^+$ , 4), 242(26), 200(30), 169(33), 157(73), 140(39), 115(100), 98(90), 82(51), and 55(28).

*4- $\beta$ -Glucopyranosyloxy-3-methyl-2-butenylnitrile (7)*. Characterized as the tetraacetate **8**.  $NH_3$  CI-MS  $m/z$  (%): 445( $[M+NH_4]^+$ , 100);  $^{15}NH_3$  CI-MS: 446( $[M+^{15}NH_4]^+$ , 100); EI-MS: 331( $[M-C_5H_6ON]^+$ , 3), 265(9), 243(15), 200(23), 169(33), 157(49), 145(50), 112(65), 98(100), 81(97), 69(45), and 53(59);  $[^1H]NMR$  (300 MHz,  $CDCl_3$ )  $\delta$  1.95 (s,  $H_3C-5$ ), 2.01–2.10 (4s, 12H), 3.72 (m, HC-3'), 4.16 (dd, HC-6'), 4.27 (dd, HC-6'), 4.48 (br s,  $H_2C-4$ ), 4.52 (d,  $J = 7.8$ , HC-1'), 5.04 (dd, HC-2'), 5.10 (dd, HC-4'), 5.22 (t, HC-3'), and 5.27 (br s, HC-2).

*Cardiospermin (2- $\beta$ -glucopyranosyloxy-3-hydroxymethyl-3-butenylnitrile) (9)*. Characterized as the pentaacetate **10**.  $NH_3$  CI-MS  $m/z$  (%): 503( $[M+NH_4]^+$ , 100); EI-MS: 331( $[M-C_7H_8O_3N]^+$ , 9), 271(2), 229(2), 211(3), 169(100), 138(19), 127(27), 109(92), 97(17), 81(15), and 69(11); IR ( $CCl_4$ ) no  $\gamma C\equiv N$ ;  $[^1H]NMR$  (300 MHz,  $CDCl_3$ )  $\delta$  2.02–2.10 (5s, 15H), 3.78 (m, HC-5'), 4.18 (dd, HC-6'), 4.27 (dd, HC-6'), 4.65 (br s,  $H_2C-5$ ), 4.83 (d,  $J = 7.8$ , HC-1'), 5.03–5.14 (m, HC-2', HC-4'), 5.20 (br s, HC-2), 5.26 (t, HC-3'), 5.34 (br s, HC-4), and 5.66 (br s, HC-4).

*4- $\beta$ -Glucopyranosyloxy-3-hydroxymethyl-2-butenylnitrile (11)*.  $NH_3$  CI-MS (probe)  $m/z$  (%): 310( $[M+(NH_3)_2H]^+$ , 42), 293( $[M+NH_4]^+$ , 100), 215( $[hexose+(NH_3)_2H]^+$ , 26), and 198( $[hexose+NH_4]^+$ , 15);  $^{15}NH_3$  CI-MS: 312(92), 294(100), 217(78), and 199(86);  $ND_3$  CI-MS: 322(39), 302(100), 227(55), and 207(55);  $CH_4$  CI-MS: 276( $[M+H]^+$ , 2); isobutane CI-MS: 332( $[M+C_4H_9]^+$ , 57), and 276(100); EI-MS: 155(2), 114(8), 97(16), 85(16),

73(72), 67(68), 60(100), and 57(51); IR(CCl<sub>4</sub>):  $\gamma$ C $\equiv$ N at 2228 cm<sup>-1</sup>. Further characterized as pentaacetate **12**. NH<sub>3</sub> CI-MS *m/z* (%): 503([M+NH<sub>4</sub>]<sup>+</sup>, 100); EI-MS: 331(2), 323(4), 292(2), 250(4), 242(11), 200(17), 169(25), 157(40), 145(37), 138(100), 115(45), 112(54), 98(58), 81(68), 70(34), 69(35), 61(7), and 52(6); [<sup>1</sup>H]NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  2.01–2.14 (5s, 15H), 3.73 (m, HC-5'), 4.17 (dd, HC-6'), 4.26 (dd, HC-6'), 4.55(d, *J* = 8.1, HC-1'), 4.59 (br s, H<sub>2</sub>C-4), 4.74 (t, H<sub>2</sub>C-5), 5.02 (dd, HC-2'), 5.09 (dd, HC-4'), 5.21 (t, HC-3'), and 5.54 (br s, HC-2); [<sup>13</sup>C]NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  20.0–20.2 (5C, CH<sub>3</sub>COO), 61.2 (C-6'), 62.3 (C-4), 66.6 (C-5), 67.7 (C-4'), 70.5 (C-2'), 71.8 (C-3' or 5'), 72.2 (C-3' or 5'), 97.2 (C-2), 99.6 (C-1'), 114.3 (C-1), 155.8 (C-3), and 168.8–170.1 (5C, CH<sub>3</sub>COO). As the shift for the nitrile carbon (C-1) was at variance with that reported by Braekman et al. (1982) ( $\delta$  120.7), a [<sup>13</sup>C]NMR (60 MHz, CDCl<sub>3</sub>) spectrum of **12** derived from *L. isolata* supplied to us by Dr. Dalozé was obtained on our instrument; the nitrile signal occurred at  $\delta$  114.6. EI- and CI-MS of **12** from *L. isolata* were identical to **12** derived from *Jadera*.

*6-O- $\beta$ -Glucopyranosyl-3 $\beta$ -glucopyranosyloxy-3-methyl-butyronitrile (13)*. Characterized as heptaacetate **14** (2.6 mg, mp = 197–197.5°C). NH<sub>3</sub> CI-MS *m/z* (%): 735([M+NH<sub>4</sub>]<sup>+</sup>, 100); ND<sub>3</sub> CI-MS: 739(100); CH<sub>4</sub> CI-MS: 746([M+C<sub>2</sub>H<sub>7</sub>]<sup>+</sup>, 9), 718([M+H]<sup>+</sup>, 17), 619([dihexose]<sup>+</sup>, 33), and 331([hexose]<sup>+</sup>, 100); EI-MS: 677([M-CH<sub>2</sub>CN]<sup>+</sup>, 1) 370(2), 331(59), 317(16), 271(8), 215(11), 169(100), 157(15), 127(20), 109(51), 97(21), 81(48), 69(29), and 55(25); [<sup>1</sup>H]NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  1.39 and 1.40 (2s, H<sub>3</sub>C-4 and H<sub>3</sub>C-5), 2.00–2.10 (7s, 21H), 2.56 (d, *J* = 2.4, H<sub>2</sub>C-2), 3.65 (d, HC-6'), 3.61–3.75 (m, HC-5', HC-5''), 3.81 (dd, HC-6'), 4.12 (dd, HC-6''), 4.27 (dd, HC-6''), 4.57 (d, *J* = 7.8, HC-1'), 4.70 (d, *J* = 8.1, HC-1''), 4.85–5.09 (m, 4H; HC-4', -4'', -2', -2''), and 5.16–5.24 (m, HC-3', HC-3''). [<sup>1</sup>H]NMR (200 MHz, CDCl<sub>3</sub>) of 3-methyl-3-(trimethylsiloxy)-butyronitrile lit. (Imi et al., 1987): 0.15 (s, 9H), 1.41 (s, 6H), and 2.47 (s, 2H).

*Cyanogenesis*. When five larvae and five adults of *J. haematoloma* reared on *K. paniculata* seeds were crushed separately in 4-ml vials, no HCN was detected even after addition of H<sub>2</sub>SO<sub>4</sub>. Single *J. haematoloma* larvae fed either *K. paniculata* or *C. grandiflorum* seeds were crushed in vials containing indicator paper and, after 10–15 min 40  $\mu$ l of a  $\beta$ -glucosidase solution was added. Bugs reared on *K. paniculata* seeds showed no indication of HCN, and addition of  $\beta$ -glucosidase did not produce HCN. Larvae fed *C. grandiflorum* seeds tested negatively for HCN at first, but positively after addition of  $\beta$ -glucosidase. Urine (ca. 3  $\mu$ l) collected from larvae fed *C. grandiflorum* was not cyanogenic even when  $\beta$ -glucosidase was added, nor was urine from larvae fed golden rain tree seeds. Last-instar *J. sanguinolenta* larvae were also tested as just described; *Cardiospermum*-fed bugs tested positively for HCN upon addition of  $\beta$ -glucosidase, but *K. paniculata*-fed bugs were acyanogenic within the observational time period (ca. 30 min).

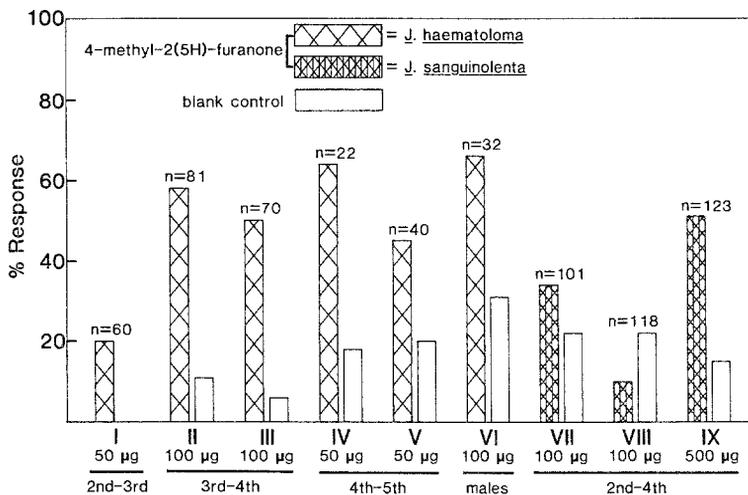
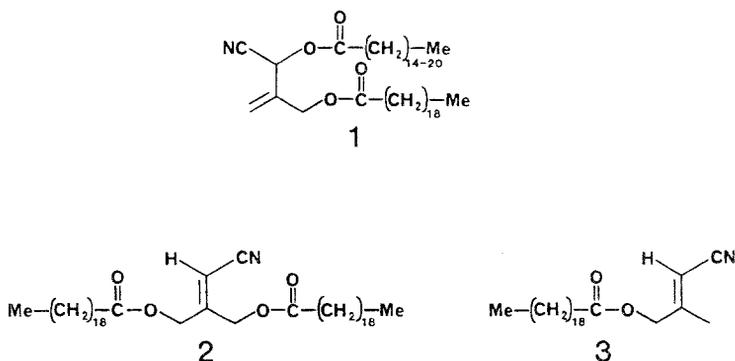


FIG. 3. Attraction of *Jadera* to 4-methyl-2(5H)-furanone.

**Attraction Bioassay.** *J. haematoloma* were attracted to **4** at the 50- and 100- $\mu\text{g}$  dosages (Figure 3, tests I-VI;  $X_1^2 > 3.84$ ,  $p < 0.05$ ). Maximum response was usually recorded the morning after the test was started. For test I the maximum response occurred 2 hr after starting the test, but by the next morning the numbers of larvae in the treated versus control arms were not significantly different and the overall response was the lowest of all the *J. haematoloma* tests. Responses of *J. sanguinolenta* larvae to **4** were low at the 100- $\mu\text{g}$  dosage and equivocal; in test VII the response was not significant from the control, and in test VIII more larvae occurred in the control arm (Figure 3). At a dose of 500  $\mu\text{g}$ , *J. sanguinolenta* larvae were significantly attracted to **4** (Figure 3, test IX;  $X_1^2 = 25.00$ ).



SCHEME 1.

*Predator Aversion.* Naive toads ate all 40 larvae offered, but later ate only two of 40 larvae and four of 40 adults (while readily eating mealworms) (Ribiero, 1989). Naive toads that initially ate adult bugs did not later avoid adults: 22 of 32 were eaten. However, toads ingesting adults in the second trial wiped their mouths and eyes and attempted to regurgitate. In tests with five captive, naive blue jays, each bird ate some or all of the three to four late-stage larvae offered initially, but only two ate bugs in a second trial; in a third trial, neither of these two birds would feed on bugs. One bird regurgitated after eating bugs. Experienced birds often tossed the bugs out of their cages. *Jadera* larvae offered to praying mantids were grasped and mandibulated for <5 sec and then thrown from the tree. Three larvae showed signs of pronotal damage, but none were permanently harmed. All five rejected larvae-emitted scent gland secretion and none of the mantids would accept another bug.

#### DISCUSSION

Serinethines are vividly marked, red insects that suck the seed oil of toxic Sapindales. They lack the stink glands common to most adult Heteroptera (hence the misnomer), yet still secrete, excrete, and bleed an impressive array of semi-chemicals. A predator faces irritating vapors of  $\alpha,\beta$ -unsaturated aldehydes, keto-aldehydes, and monoterpenes and, if it persists, is likely to have its mouth smeared with blood containing cyanogenic glucosides. For color-blind predators like shrews (Insectivora) (Huheey, 1984), scent gland odors may serve as aposematic signals associated with bloodborne toxins.

Our results show that HCN is released from crushed *Jadera*, but only in the presence of a  $\beta$ -glucosidase and only if the bugs are reared on balloon vine seeds. Sequestration of the glucoside cardiospermin by *Jadera* contrasts strikingly with the absence of this compound from the blood of lycaenid caterpillars that feed on balloon vine in which the concentration of cardiospermin is high (cf. Braekman *et al.*, 1982). Glucosides accumulated in the blood of *Jadera* fed soapberry or golden rain tree seeds are not truly cyanogenic; however, even acyanogenic cyanolipids exhibit insecticidal activity (Mikolajczak *et al.*, 1984), and many predators reject bugs containing glucosides of these compounds.

The specialized *Jadera* mode of life is further highlighted by the ease with which the bugs exude blood. This capability is also reported for the serinethine *L. isolata* (Daloze *et al.*, 1982) and is well known for *Oncopeltus fasciatus* (Heteroptera: Lygaeidae) and certain other lygaeine seed predators of cardenolide-containing Apocynales (Scudder *et al.*, 1986). Notwithstanding the congeneric status of *L. isolata* and *L. trivittatus* (the boxelder bug), the latter species failed to ooze blood even after very rough handling (Aldrich, unpublished). In

fact, the glycoside chemistry of the former species is much more closely allied to that of *Jadera* than boxelder bugs. Certainly this is because *L. isolata* and *Jadera* feed on sapindaceous plants.

Analysis of the excreta of *J. haematoloma* and *J. sanguinolenta* for volatiles revealed a heretofore unknown dietary effect with intriguing ecological ramifications. 4-Methyl-2(5H)-furanone (**4**) is excreted by adults and larvae only if the bugs feed on golden rain tree (or soapberry?) seeds, and the bugs are attracted to this lactone. In southwestern North America, soapberry and golden rain trees typically mature thousands of seeds per tree at once in August and September, compared to the more continuous maturation of usually no more than several hundred seeds for balloon vine in the Florida Keys (Carroll, 1988). These phenological idiosyncrasies apparently have been paramount for evolution of *J. haematoloma* adults in Florida that are larger and produce larger eggs that take longer to hatch than do *Jadera* from Oklahoma (Carroll, 1988). For populations of Serinethinae on soapberry and golden rain trees, the limiting "resource" may be time rather than food (Carroll, 1988), so the orientation of *Jadera* individuals to a volatile excretory product could be an adaptive response to save time.

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#### REFERENCES

- ALDRICH, J.R. 1988. Chemical ecology of the Heteroptera. *Annu. Rev. Entomol.* 33:211–238.
- ALDRICH, J.R., BLUM, M.S., LLOYD, H.A., EVANS, P.H., and BURKHARD, D.R. 1979. Novel exocrine secretions from two species of scentless plant bugs (Hemiptera: Rhopalidae). *Entomol. Exp. Appl.* 26:323–331.
- BRAEKMAN, J.C., DALOZE, D., and PASTEELS, J.M. 1982. Cyanogenic and other glucosides in a neo-Guinean bug *Leptocoris isolata*: Possible precursors in its host-plant. *Biochem. Syst. Ecol.* 10:355–364.
- CARROLL, S.P. 1988. Contrasts in reproductive ecology between temperate and tropical populations of *Jadera haematoloma*, a mate-guarding hemipteran (Rhopalidae). *Ann. Entomol. Soc. Am.* 81:54–63.
- CARROLL, S.P., and LOYE, J.E. 1987. Specialization of *Jadera* species (Hemiptera: Rhopalidae) on the seeds of Sapindaceae (Sapindales), and coevolutionary responses of defense and attack. *Ann. Entomol. Soc. Am.* 80:373–378.
- DALOZE, D. BRAEKMAN, J.C., and PASTEELS, J.M. 1982. Cyanogenic and other glucosides from a neo-Guinean bug *Leptocoris isolata* (Heteroptera: Rhopalidae). *Les Mediateurs Chim.* 7:141–145.

- FEIGL, F. 1966. Spot Tests in Organic Analysis. Elsevier, New York. 547 pp.
- HUHEEY, J.E. 1984. Warning coloration and mimicry, pp. 257-297, in W.J. Bell, and R.T. Cardé (eds.). Chemical Ecology of Insects. Chapman and Hall, London.
- IMI, K., YANAGIHARA, N., and UTIMOTO, K. 1987. Reaction of cyanotrimethylsilane with oxiranes. Effects of catalysts or mediators on regioselectivity and ambient character. *J. Org. Chem.* 52:1013-1016.
- LIARDON, R., and PHILIPPOSIAN, G. 1978. Volatile components of fermented soya hydrolysate. *Z. Lebensm. Unters. Forsch.* 167:180-185.
- MIKOLAJCZAK, K.L., SMITH, C.R., JR., and TJARKS, L.W. 1970. Cyanolipids of *Koelreuteria paniculata* Laxn. seed oil. *Lipids* 5:672-677.
- MIKOLAJCZAK, K.L., MADRIGAL, R.V., SMITH, C.R., JR., and REED, D.K. 1984. Insecticidal effects of cyanolipids on three species of stored product insects, European corn borer (Lepidoptera: Pyralidae) larvae, and striped cucumber beetle (Coleoptera: Chrysomelidae). *J. Econ. Entomol.* 77:1144-1148.
- PELLETIER, S.W., DJARMATI, Z., LAJSIC, S.D., MICOVIC, I.V., and YANG, D.T.C. 1975. A general method for alkylating 2(5H)-furanones; Reduction of products to 3-, 2,3-, and 3,4-substituted furans. *Tetrahedron* 31:1659-1665.
- PRICE, C.C., and JUDGE, J.M. 1973.  $\gamma$ -Crotonolactone. *Org. Syn. Collect.* 5:255-258.
- RIBEIRO, S. T., 1989. Group effects and aposematism in *Jadera haematoloma* (Hemiptera: Rhopalidae). *Ann. Entomol. Soc. Am.* 82:466-475.
- SCHAEFER, C.W., and CHOPRA, N.P. 1982. Cladistic analysis of the Rhopalidae, with a list of food plants. *Ann. Entomol. Soc. Am.* 75:224-233.
- SCHAEFER, C.W., and MITCHELL, P.L. 1983. Food plants of the Coreidea (Hemiptera: Heteroptera). *Ann. Entomol. Soc. Am.* 76:591-615.
- SCUDDER, G.G.E., MOORE, L.V., and ISMAN, M.B. 1986. Sequestration of cardenolides in *Oncopeltus fasciatus*: Morphological and physiological adaptations. *J. Chem. Ecol.* 12:1171-1187.
- SEIGLER, D.S. 1974. Determination of cyanolipids in seed oils of the Sapindaceae by means of their NMR spectra. *Phytochemistry* 13:841-843.
- SEIGLER, D.S., and KAWAHARA, W. 1976. New reports of cyanolipids from sapindaceous plants. *Biochem. Syst. Ecol.* 4:263-265.
- SEIGLER, D.S., MIKOLAJCZAK, K.L., SMITH, C.R., JR., and WOLFF, I.A. 1970. Structure and reactions of a cyanogenetic lipid from *Cardia verbenacea* DC seed oil. *Chem. Phys. Lipids* 4:147-161.
- SEIGLER, D.S., EGGERDING, C., and BUTTERFIELD, C. 1974. A new cyanogenic glycoside from *Cardiospermum hirsutum*. *Phytochemistry* 13:2330-2332.
- SPENCER, G.F., and DAXENBICHLER, M.E. 1980. Gas chromatography-mass spectrometry of nitriles, isothiocyanates and oxazolidinethiones derived from cruciferous glucosinolates. *J. Sci. Food Agric.* 31:359-367.
- WOLDA, H., and TANAKA, S. 1987. Dormancy and aggregation in a tropical insect *Jadera obscura* (Hemiptera: Rhopalidae). *Proc. Kon. Nederl. Akad. Wetenschappen* 90C:351-366.