9 Villar, A., Gascó, M. A., Alcaraz, M. J., Máñez, S., and Cortes, D., Planta Med. *51* (1985) 70.
10 Payá, M., Máñez, S., and Villar, A., Z. Naturforsch. *41c* (1986) 976.
11 Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. biol. Chem. *193* (1951) 265.
12 Mansuy, D., Sassi, A., Dansette, P. M., and Plat, M., Biochem. biophys. Res. Commun. *135* (1986) 1015.
13 Osawa, T., Ide, A., Su, J.-D., and Namiki, M. J., Agric. Food Chem. *35* (1987) 808.
14 Scott, R., and Slater, T. F., in: Recent Advances in Lipid Peroxidation and Tissue Injury, p. 233. Eds T. F. Slater and A. Garner. Brunel University Printing Services, Uxbridge 1981.
15 Ratty, A. K., and Das, N. P., Biochem. Med. Metab. Biol. *39* (1988) 69.
16 Younes, M., and Siegers, C. P., in: Free Radicals in Liver Injury, p. 87. Eds G. Poli, K. H. Cheeseman, M. U. Dianzani and T. F. Slater. IRL Press, Oxford 1985.
17 Afanas'ev, I. B., Dorozhko, A. I., Brodskii, A. V., Kostyuk, V. A., and Potapovitch, A. I., Biochem. Pharmac. *38* (1989) 1763.
18 Slater, T. F., and Eakins, M. N., in: New Trends in the Therapy of Liver Diseases, p. 84. Ed. A. Bertelli. Karger, Basel 1975.
19 Slater, T. F., in: Free Radicals, Lipid Peroxidation and Cancer, p. 243. Eds D. C. H. McBrien and T. F. Slater. Academic Press, London 1982.
20 Torel, J., Cillard, J., and Cillard, P., Phytochemistry *25* (1986) 383.
21 Sousa, R. L., and Marletta, M. A., Archs Biochem. Biophys. *240* (1985) 345.
22 Havsteen, B., Biochem. Pharmac. *32* (1983) 1141.
23 Campos, R., Garrido, A., Guerra, R., and Valenzuela, A., in: Plant Flavonoids in Biology and Medicine II. Biochemical, Cellular and Medicinal Properties, p. 375. Eds V. Cody, E. Middleton Jr, J. B. Harborne and A. Beretz. Alan R. Liss Inc., New York 1988.

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Insect anti-juvenile hormone and juvenile hormone activity from plants in the genus *Nama*

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Summary. The insect anti-juvenile hormones precocene I and II (7-methoxy-2,2-dimethyl-2H-1-benzopyran and 6,7-dimethoxy-2,2-dimethyl-2H-1-benzopyran) were identified in three of nine *Nama* (Hydrophyllaceae) species. Precocene I occurred in *N. lobbii* while precocene II occurred in *N. hispidum, N. lobbii* and *N. sandwicense. N. hispidum* contained the highest concentration (ca 0.5% dry weight) of precocene II, which was found in the leaves, stems, seed capsules, corolla, glandular trichomes, and seeds. In addition to the anti-juvenile hormone, insect juvenile hormone activity was detected in the organosoluble extracts of *N. rothrockii* and *N. sandwicense. N. sandwicense* is the first plant discovered to contain compounds with both anti- and juvenile hormone activity.
Key words. Nama; Hydrophyllaceae; precocenes; anti-juvenile hormones; juvenile hormone activity.

Certain phytochemicals are recognized to adversely impact the endocrine system of insects by mimicking the insect's natural juvenile hormone (JH) or acting as antagonists to the production or action of JH [1,2]. Since JH regulates many important physiological functions including metamorphosis [3], an insect's life cycle can be disrupted by the presence of these phytochemicals. As a consequence of JH mimic activity, some insects may undergo abnormal growth and delayed metamorphosis [4]. When JH is eliminated, due to JH antagonists or anti-juvenile hormone (AJH) activity, some insects may prematurely molt to adults, enter diapause, or become sterilized [5]. Even minute amounts of these plant compounds are often sufficient to disrupt the insect's physiology and development [6].

Compounds with juvenile hormone activity have been isolated from a variety of plants [6,7], while AJHs have been previously isolated only from the Asteraceae [5,8]. These types of compounds have never been reported to occur simultaneously in the same plant. We have now discovered in plants in the genus *Nama* (Hydrophyllaceae) compounds which disrupt the endocrine system of the large milkweed bug, *Oncopeltus fasciatus. Nama hispidum* contained the AJH, precocene II (6,7-dimethoxy-2,2-dimethyl-2H-1-benzopyran) while *N. rothrockii* had at least two compounds with JH activity. *N. sandwicense* contained precocene II (PII) as well as JH activity. The distribution and concentration of P II and the related compound P I (7-methoxy-2,2-dimethyl-2H-1-benzopyran) are reported for the nine *Nama* species collected and for the different anatomical parts of *N. hispidum.*

Experimental

Plant material. Specimens in the genus *Nama* were collected during the spring and summer of 1986 at the following localities: *N. demissum* Gray: Clark Co., Nevada, USA; *N. densum* Lemmon: Mono Co., California, USA; *N. hispidum* Gray: Pima Co., Arizona, USA; *N. jamaicense* Linn: Dominican Republic; *N. lobbii* Gray: Eldorado Co., California, USA; *N. rothrockii*: Inyo Co., California, USA; *N. sandwicense* Gray: Maui Co., Hawaii, USA; *N. stevensii* Hitchcock: Eddy Co., New

Mexico, USA; *N. xylopodum* (Woot. and Standl.): Eddy Co., New Mexico, USA. Voucher specimens for the species collected in Arizona, California, Nevada, and New Mexico were identified by the authors and are deposited at the University of Arizona herbarium. *N. sandwicense* was generously provided by Dr Evangeline Funk, University of Hawaii. *N. jamaicense* was a gift from Dr Domenica Abramo, Department of Chemistry, Universidad Catolica Madre y Maestra, Santiago, Dominican Republic and was identified by Dr Thomás Zanoni, Jardin Botanico National, Santo Domingo, Dominican Republic.

Biological evaluations. 20 second instar nymphs of the large milkweed bug *Oncopeltus fasciatus* were exposed to plant extract residues or chromatographic fractions as previously described[5]. Crude extracts were assayed at 80, 40, 20 μg/cm^2. Column fractions were assayed at 80 μg/cm^2. Anti-juvenile hormone activity was scored for precocious adultoids after 12 days[5]. Juvenile hormone activity was scored if the insects entered a supernumerary 6th instar[4].

Reference compounds. Synthetic precocene I and II[5,9] were used as standards.

Instrumentation and general methods. Extraction: Finely ground, air dried plant material from each *Nama* species was extracted by steeping overnight in CH_2Cl_2. Open column chromatography: following removal of the CH_2Cl_2 under reduced pressure, 1 g of crude extract was applied to 30 g Florisil (deactivated with 7% water by weight) and eluted with an ascending series of diethyl ether: hexane[10]; gas chromatography/mass spectrometry (GC/MS): a sample of each fraction from the above elution series was injected on a 12 m × 0.25 mm HP1 methyl silicone column. Gas flow rate for He: 1.0 ml/min. Temperature program: 100 °C for 3 min then to 250 °C at 20 °C/min. EI: 70 eV. The synthetic precocenes and plant antihormones were detected using selected ion monitoring (SIM): m/z = 175 for PI and m/z = 205 for PII[11].

Results and discussion

When exposed to crude extracts of *Nama rothrockii* or *N. sandwicense*, nymphs of the large milkweed bug *Oncopeltus fasciatus* molted into a supernumerary instar and eventually died. Open column fractionation of the *N. rothrockii* crude extract yielded two fractions with JH activity. An apolar compound was found in one fraction while several polar compounds were included in the second fraction[12]. Separations of *N. sandwicense* extract indicate that it may also contain more than one juvenile hormone mimic. It is unclear whether the JH active compounds of *N. rothrockii* are structurally related to the active components in *N. sandwicense*. A complete structural analysis of these JH mimics from *N. rothrockii* and *N. sandwicense* is in progress.

In contrast to nymphs of *O. fasciatus* treated with extracts of *N. rothrockii* and *N. sandwicense*, nymphs treated with crude extracts of *N. hispidum* matured precociously to diminutive sterile adultoids. After fractionation of this extract on Florisil and analysis by GC/MS, the anti-juvenile hormone causing precocious metamorphosis was identified as precocene II by comparison with authentic standards. The antihormonal activity associated with this compound was previously discovered only in extracts of plants from the genus *Ageratum* (Asteraceae), which is phylogenetically distant from *Nama*[13].

Table 1. Insect hormonal activity and precocene level in *Nama* extracts

Species	Hormonal activity	Precocenes⁺ (mg/g)	
		PI	PII
N. demissum	*	0	0
N. densum	*	0	0
N. hispidum var. *gypsicola*	anti-JH	0	0.3
N. hispidum var. *revolutum*	anti-JH	0	69.1
N. jamaicense	*	0	0
N. lobbii	*	Trace†	Trace
N. rothrockii	JH	0	0
N. sandwicense	JH	0	1.4
N. stevensii	*	0	0
N. xylopodum	*	0	0

*No hormonal activity. ⁺Detected by GC/MS (PI: m/z 175; PII: m/z 205), limit 100 μg/g. †Less than 0.2 mg/g.

Nine *Nama* species were evaluated by GC/MS for their content of precocene II and precocene I, with selected ion monitoring (SIM) (table 1). Precocene II (m/z 205) was detected in *N. hispidum, N. lobbii* and *N. sandwicense*, while precocene I (m/z 175) was detected only in *N. lobbii.* Replicated bioassays on *O. fasciatus* showed that *N. lobbii* had no anti-juvenile hormone activity while *N. sandwicense* retained only juvenile hormone activity. Compounds possessing JH activity can overcome the anti-JH action of PI and PII[14], suggesting that the *N. sandwicense* JH mimic may have masked the effects of the anti-juvenile hormone. Importantly, *N. sandwicense* is the first plant discovered to possess compounds with AJH and JH activity.

There was considerable variation among the *Nama* species in the levels of PI and PII, which may account for the lack of observable anti-JH activity of extracts in some of the species (table 1). Precocene II was over 0.5% (table 2) of the dry weight of *N. hispidum* but only 0.005% (0.046 mg/g plant) of the dry weight of *N. sandwicense.* Trace amounts (less than 0.2 mg/g extract) of both precocene I and II were detected in *N. lobbii* but neither precocene I nor precocene II was detected in *N. demissum, N. densum, N. jamaicense, N. rothrockii, N. stevensii,* or *N. xylopodum.* Because *O. fasciatus* nymphs are susceptible to contact doses of PII as low as 0.7 μg/cm^2, the trace amounts of the antihormone in the *N. lobbii* extract were insufficient to provoke an observable effect on the test animals[5].

Within-species variation of precocene II was also large in *N. hispidum. N. hispidum* var. *revolutum* from Tucson, Arizona, had a uniformly high concentration of precocene II but *N. hispidum* var. *gypsicola* from New Mex-

Table 2. Precocene II content of two varieties of *Nama hispidum*

Structure	Variety	
	revolutum (Arizona)	*gypsicola* (New Mexico)
Whole plant	5.3*	0.008
Corolla	0.875	–
Capsule	0.448	0.006
Seed	0.026	ND
Seedling	2.7	ND

*Expressed as mg PII/g dry plant. ND = Not detected by GC/MS, 100 μg/g of crude extract.

ico had much lower amounts of anti-juvenile hormone (table 2). The variation may result from genetic differences between the populations (i.e. characterized as varieties in *N. hispidum*) or from responses to variable environmental factors. Each plant population has characteristic habitats, varied exposure to precipitation, distinct phenologies, and differences in herbivore predation. Most notable was the disparate predator load. All the plants collected from New Mexico were heavily attacked by insect herbivores while the plants from Tucson were seemingly untouched by any plant predator. Apparently, the plants from Tucson, which contain high concentrations of precocene II, are far less acceptable to herbivores.

In *N. hispidum* collected from Tucson, precocene II was detected in whole plants, seed capsules, corolla, and seeds (table 2). Moreover, laboratory-reared seedlings contained high amounts of precocene II which indicates that *N. hispidum* rapidly synthesized this anti-juvenile hormone after germination. Precocene II was also detected in the densely packed glandular trichomes found on the stems, seed capsules, and adaxial and abaxial surface of the leaves and sepals. Since glandular trichomes are recognized to protect plants from some insect herbivores[15,16], these structures may provide some protection in *N. hispidum* seedlings and mature plants.

Precocene I and II probably reduce the number of insect attacks to *N. hispidum* by interfering with insect development, reproduction, and communication[17]. Moreover, precocene II has antifeedant activity[18] and the ability to inhibit digestion[12,19]. Even a small dose of precocene II has a profound physiological effect on many insects[17]. The broad range of biological activities of the precocenes could help protect *N. hispidum* and other species in the genus against a diverse array of phytophagous insects. Moreover, because precocene I and II have now been found in a plant family other than the Asteraceae, we believe that the precocenes may have a wide-spread role in angiosperm phytochemical defense against herbivores. Anti-juvenile hormones also might complement other biologically active phytochemicals commonly found in the Asteraceae[20] and in the Hydrophyllaceae[21]. Plants like *Nama sandwicense*, harboring a multitude of these compounds, might have simultaneous resistance to a variety of predators and pathogens. Certainly, the chemical diversity of *Nama* indicates that this genus deserves further attention as a potential source of new and interesting compounds active against the insect endocrine system.

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1 Bowers, W. S., Insect-plant interactions: endocrine defenses, in: Origins and Development of Adaptation, p. 119. Eds D. Evered and G. M. Collins. Pitman, London 1984.
2 Horn, D. H. S., and Bergamasco, R., in: Comprehensive Insect Physiology, Biochemistry, and Pharmacology, vol. 7, p. 185. Eds G. A. Kerkut and L. I. Gilbert. Pergamon Press, Oxford 1985.
3 Wigglesworth, V. B., in: Comprehensive Insect Physiology, Biochemistry, and Pharmacology, vol. 7, p. 1. Eds. G. A. Kerkut and L. I. Gilbert. Pergamon Press, Oxford 1985.
4 Bowers, W. S., Lipids *13* (1978) 736.
5 Bowers, W. S., Ohta, T., Cleere, J. S., and Marsella, P. A., Science *193* (1976) 542.
6 Nishida, R., Bowers, W. S., and Evans, P. H., Archs. Insect Biochem. Physiol. *1* (1983) 17.
7 Bowers, W. S., Fales, H. M., Thompson, M. J., and Uebel, E. C., Science *154* (1966) 1020.
8 Alertsen, A. R., Acta chem. Scand. *9* (1955) 1725.
9 Camps, F., Coll, J., Messeguer, M. A., Pericas, S., Ricart, S., Bowers, W. S., and Soderlund, D. M., Synthesis *9* (1980) 725.
10 Carroll, K. K., J. Lipid Res *2* (1961) 135.
11 Ekondayo, O., Laakso, I., Hiltunen, R., and Seppänen, T., J. Chromat. *403* (1987) 358.
12 Binder, B. F., Thesis, University of Arizona, Tucson, Arizona (1989).
13 Cronquist, A., An Integrated System of Classification of Flowering Plants. Columbia University Press, New York 1981.
14 Bowers, W. S., Am. Zool. *21* (1981) 737.
15 Lin, S. Y. H., and Trumble, J. T., Ent. exp. appl. *41* (1986) 53.
16 Juniper, B. D., and Southwood, T. R. E., Insects and the Plant Surface. Edward Arnold, Ltd., Baltimore 1986.
17 Bowers, W. S., in: Comprehensive Insect Physiology, Biochemistry, and Pharmacology, vol. 8, p. 551. Eds G. A. Kerkut and L. I. Gilbert. Pergamon Press, Oxford 1985.
18 Azambuja, P. D., Bowers, W. S., Ribeiro, J. M. C., and Garcia, E. S., Experientia *38* (1982) 1054.
19 Binder, B. F., and Bowers, W. S., J. Electron Microsc. Tech. *11* (1989) 93.
20 Heywood, V. H., Harborne, J. B., and Turner, B. C., The Biology and Chemistry of the Compositae. Academic Press, New York 1977.
21 Kelsey, R. G., Reynolds, G. W., and Rodriguez, E., in: Biology and Chemistry of Plant Trichomes, p. 187. Eds E. Rodriguez, P. L. Healey and I. Mehta. Plenum Press, New York 1984.

0014-4754/91/020199-03$1.50 + 0.20/0