Journal of Chemical Ecology, Vol. 21, No. 3, 1995

ON INSECT ATTRACTANTS FROM PITCHER PLANTS OF THE GENUS *Heliamphora* (SARRACENIACEAE)

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(Received January 20, 1994; accepted December 1, 1994)

Abstract—Examination of *Heliamphora heterodoxa* and *H. tatei* from the Guayana Highlands of Venezuela reveals that the enol diacetal monoterpene, sarracenin, is the major volatile compound present in the spoon-shaped structures of leaves of the pitchers. In addition, erucamide, phenol, cinerone, phenylacetaldehyde, and a series of methyl esters also occur in extracts of the spoon-shaped appendages of pitchers at the time during which they attract insects.

Key Words—*Heliamphora*, carnivorous plants, pitcher plants, sarracenin, insect attractants.

INTRODUCTION

Carnivorous plants are an extreme example of the diversity of insect–plant relationships (Darwin, 1888). The arthropod prey is attracted, caught, and degraded by specially adapted leaves supplementing the limited amount of nutrients available to these plants (Lloyd, 1942; Rymal and Folkerts, 1982; Juniper et al., 1989).

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0098-0331/95/0300-0379$07.50/0

Plants in the genus *Heliamphora* Benth. (Sarraceniaceae) endemic to the Precambrian sandstone formations ("Tepuy") in Venezuela were not included by Darwin (1888) in the first extensive revision of carnivorous plants, and only recently have they been studied in some detail (Jaffé et al., 1992). These plants possess visual cues (Joel et al., 1985) in addition to special leaf appendages producing chemical secretions in order to attract their prey to the pitcher (Jaffé et al., 1992). Brewer-Carias (1972) was the first to describe the feeding behavior of the plant, reporting that a honeylike fluid produced by nectaries in the spoon-shaped appendage of the leaves attracted a variety of insects.

The macroscopic anatomy of a *Heliamphora* pitcher has been described in considerable detail (Lloyd, 1942). The transformed leaf or pitcher has a nectary or spoon, a bell zone, an intermediary constriction, and a funnel or tank (Jaffé et al., 1992). *Heliamphora* leaves have a reddish coloration on the spoon and the surrounding area, which may further act to attract potential prey items. UV light-reflecting guides also have been reported on *Heliamphora* leaves that probably enhance the attraction of certain insect groups (Joel et al., 1985; Juniper et al., 1989). Sun-exposed *Heliamphora* plants have pitchers of a generally constant size within a given species. They have well-developed spoons that are conspicuously red colored and fragrant. However, in instances where pitcher plants grow in the shade of crevasses, shrubs, or small trees, the appearance of the plants is very different. These pitchers are bright green without the red pigment, the hairs in the upper part of the pitcher are lost, and the size of the spoon is reduced. The bell or upper part of the leaf increases while the tank is reduced, becoming thinner and resulting in the pitcher being larger and more slender (Jaffé et al., 1992).

Pitcher leaves have an optimum age for attracting and capturing prey. For example, we know from field observations that fully developed leaves capture the largest number of prey items (arthropod carcasses) compared to very young or very old leaves (Jaffé et al., 1992).

Heliamphora pitchers attract and trap a large variety of arthropod species. Prey is apparently attracted to the spoon-shaped appendage at the tip of each pitcher, which accumulates a sweet-smelling and highly palatable sticky secretion. The odor of the secretion varies according to the location (Tepuy) or species. This secretion is very attractive to insects in various orders, but ants seem to constitute the main prey items (Gonzales et al., 1991; Jaffé et al., 1992).

The present study identifies several of the chemicals produced by the spoon-like leaf appendage in plants actively attracting insects.

METHODS AND MATERIALS

Nectaries of *Heliamphora heterodoxa* Steyermark were obtained at Auyantepuy, Estado Bolivar, by cutting the spoonlike leaf appendages and collecting

them in 5-ml vials with ethyl acetate or methylene chloride. For laboratory studies, we collected plants of *H. heterodoxa* at Auyantepuy, whereas those of *H. tatei* Gleason were from Aracamuni, Estado Amazonas, Venezuela. Nectaries from plants maintained in the laboratory were collected monthly by placing them in glass vials containing 5 ml of either ethyl acetate or methylene chloride. Each extract was concentrated to approximately 0.5 ml using a gentle nitrogen stream, and 1 μl of each extract was injected into the gas chromatograph (GC) or gas chromatograph–mass spectrometer (GC-MS).

GC and GC-MS analyses were performed in two different laboratories. The first GC-MS data identifying sarracenin were obtained on an LKB-2091 mass spectrometer equipped with a Shimadzu model GC-9A gas chromatograph using a J & W Instrument Co. 20-m × 0.18-mm-ID capillary column coated with their DB-5 methyl silicone. The column was programmed from 50 to 270°C at 10°C/min. The ion chamber was set at 270°C and spectra were run at 70 eV. Other GC-MS data in this laboratory came from a Hewlett-Packard 5890A gas chromatograph programmed from 50 to 275°C at 7°C/min using a 30-m Restec RTX-1 dimethylsiloxane capillary column 0.25 mm ID and 0.25 μm film thickness with data collected on a Finnigan Ion Trap Mass Spectrometer (ITMS) using helium as a buffer gas at 150°C. Sarracenin was also isolated here from the nectary extracts by preparative TLC on silica gel using 50:50 toluene–ethyl acetate, its identity being confirmed by GC-MS on the LKB-2091 GC-MS system.

In the other laboratory, samples from *H. heterodoxa* and *H. tatei* plants grown in the laboratory and *H. heterodoxa* grown in the field were analyzed using a Hewlett Packard model 5971A Mass Selective Detector (GC-MS) fitted with a HP 5908 Series II gas chromatograph and an MS-Chemstation data system. The instrument was operated in scan mode at an ionization energy of 70 eV. The temperature program and column were the same as described above.

In order to evaluate qualitatively the attractiveness of spoons (nectaries) of *H. heterodoxa* and *H. tatei* growing in the laboratory, they were cut from the plants and placed in a closed 1-m^3 chamber. Five house flies were released in the chamber and their landing sites observed for over 2 hr. Spoons were also placed on the foraging table of a *Solenopsis geminata* ant colony, and the number of workers recruited to the spoon were recorded.

RESULTS

Analyses of *H. heterodoxa* spoons from field collections revealed the presence of a number of chemical compounds. Sarracenine (an iridoid) was identified using all of the above systems by comparison with mass spectral data reported by Miles et al. (1976). Figure 1 (top) (from *H. tatei* run on the Hewlett Packard 5971A spectrometer) shows a typical chromatogram. Direct comparison of the retention time and mass spectrum was made using the LKB-2091 GC-MS system

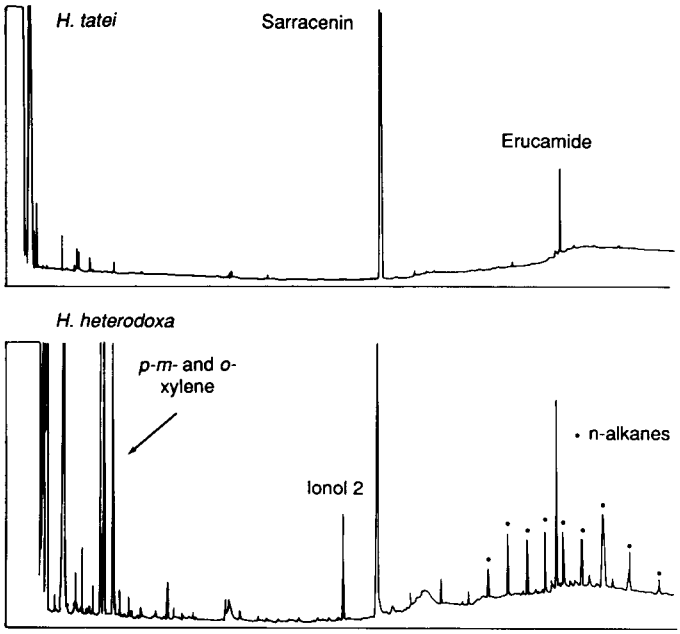

FIG. 1. Gas chromatograms of extracts of attractive spoons from *Heliamphora* pitchers. Samples of *H. tatei* came from CH_2Cl_2 extracts of pitchers growing in the laboratory; the sample of *H. heterodoxa* was an ethyl acetate extract from pitchers collected in the field.

using a sample of sarracenin kindly supplied by Professor J.K. Whitesell, Department of Chemistry, University of Texas. In addition, a sample of sarracenin purified by TLC was examined by gas chromatography–high-resolution mass spectrometry (Edward Larkin, University of Minnesota Mass Spectrometry Service Laboratory) giving a molecular ion at *m/z* 226.0854 (sarracenin $C_{11}H_{14}O_5$, requires 226.0867). Erucamide (13-docosenamide) also seen in Figure 1 as a late-eluting peak, was identified by comparison with its mass spectrum in the Wiley data base. Figure 1 (bottom) also reveals the presence of *o*-, *m*-, and *p*-xylene, 2,6-di-*tert*-butyl 4-ethylphenol (ionol-2), and a series of *n*-alkanes ranging from C_{21} to C_{33}, all identified by comparison with spectra in the Wiley data base. Ionol-2 is a likely contaminant from the solvents since it is employed as a commercial antioxidant.

Further analyses of *H. tatei* on the ion trap mass spectrometer resulted in the identification of phenol and phenylacetaldehyde in approximately equal concentrations. The terpene cinerone, also a significant constituent, was easily identified by its molecular ion at *m/z* 150 and its characteristic mass spectrum with

a base peak at 41 as seen in the NIST-NIH data base (Version 4.5, US Department of Commerce, NIST, Gaithersburg, Maryland). Three esters, methyl palmitate, ethyl palmitate, and methyl linolelaidate were detected in the ethyl acetate extracts.

Using the simple landing-site bioassay (Methods and Materials) the nectaries of the leaves of *H. tatei* and *H. heterodoxa* raised in the laboratory [(as opposed to the field, Jaffé et al. (1992)] did not, in fact, attract flies or ants. GC-MS analyses of three samples of each species of these nonattractive spoons revealed that neither sarracenin nor erucamide were present in these samples. However, on one occasion we did detect nectaries that were attractive to insects. These were found on leaves of a single large *H. tatei* plant grown in the laboratory that produced a sticky secretion on the inside of the spoons similar to that observed in plants in the field (Jaffé et al., 1992). Thus, approximately one third of a spoon was removed from the plant and of 10 observed fly landings, six were on the spoon section. The same section attracted a modest group (>10) of ant workers during a 15-min period following the placement of the section on the foraging table of the ant colony. Accordingly, the remaining parts of the spoon were extracted with CH_2Cl_2 and is the source of the GC-MS in Figure 1 revealing the presence of sarracenin and erucamide. Figure 1 (bottom) shows a similar chromatogram from field-grown *H. heterodoxa* and these same two compounds are seen to be present. Unfortunately, no samples of wild growing *H. tatei* were available due to difficult and restricted access to the site.

DISCUSSION

The enol diacetal monoterpene sarracenin, first identified in leaf extracts of *Sarracenia flava* (Miles et al., 1976), is seen to be the major volatile compound from the spoon-shaped structure of leaves of *H. heterodoxa* and *H. tatei*. In addition, *o*- and *m*-xylene and phenylacetaldehyde, identified here, were also found in *S. flava* (Miles et al., 1976). Interesting among the other compounds produced by the spoon-shaped appendages of attractive pitchers was erucamide. This compound appears in the literature as an anti-block additive for inks and sticky substances (Nagura et al., 1992; Radosta and Riley, 1991), raising the possibility that plants may produce this compound to endow its nectar secretion with certain advantageous physical properties to entangle or trap insects.

The fact that sarracenin and erucamide were rarely produced in plants growing in the laboratory, and when present, accompanied attraction of insects, suggests they have a biological role. Possible functions could include attraction, trapping, killing, or anesthetizing insects; facilitating feeding on the secretion; maintaining a low viscosity of the secretion; etc. In the case of *S. flava*, Mody et al. (1976) have shown that the insect paralyzing-activity is due to coniine, and it would be interesting to try to detect this base in these plants.

We have previously shown that plants growing in the laboratory lose their attractive properties. This suggests that the morphological changes plants suffer when they lack light in the field (Jaffé et al., 1992) are equivalent to those they suffer when growing in the laboratory. This is in accord with previous proposals (Givinish et al., 1984) suggesting that carnivory is only advantageous to the plant in light-saturated habitats. If plants lose the ability to capture additional nutrients in light-poor environments, they may save energy by not producing attractive chemical secretions or undergoing costly morphological features (Jaffé et al., 1992).

It would be very interesting to know if the ability of the pitchers to attract a large variety of different arthropods (ants, moths, flies, crickets, bees, and scorpions) is due to some or all of the compounds reported in this work.

Acknowledgments—We would like to thank Solange Issa for help with some bioassays. Fran Morales, Carlos Cardenas, and Leonardo Carballo took care of the plants in the laboratory. This work was partially supported by grant BID-CONICIT QF-36.

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