ent as the (+) enantiomer. The (−) enantiomer was below the detection level, which indicates at least 90% enantiomeric excess. Our bio-assays with *Analipus* were carried out with racemic hormosirene, and a possible enantiomer-selectivity could not be evaluated. Likewise, the enantiomer specificity of ectocarpene secretion and activity is not known at present.

The results reported here on the sexual pheromone system of *Analipus* reveal a degree of elaboration not known before in algae. Although the compounds involved are relatively simple olefinic hydrocarbons, the chemo-communication system is complicated by the involvement of two compounds in a complementary manner; the high concentration of a weakly active agent is combined with a highly active molecule in low concentration. Our knowledge of the details of brown algal pheromone systems is still limited, and it seems premature to comment on the ontogenetic or phylogenetic significance of a two-component system such as that described here for *Analipus*.

The taxonomic position of *Analipus* within the brown algal system is still uncertain. Some authors have placed it in the order Chordariales, referring to anatomical similarities[11]. Nakamura[12] suggested transferring it to the newly established order Ralfsiales. Still other authors[13] dispute the value of the order Ralfsiales and refer *Analipus* to the Ectocarpales, where it occupies a rather isolated position.

The results of this study show that progress in analytical methods will make it necessary to re-evaluate earlier work, since at least some of the brown algal pheromone systems may be more complicated than was previously believed in respect of bouquet composition, relative biological activities, and enantiomer composition.

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Chemical analysis of the pheromone blends produced by males and females of the neotropical moth, *Mocis megas* (Guénée) (Lepidoptera, Noctuidae, Catocalinae)

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Summary. Pheromonal secretions produced by females and males of the noctuid moth, *Mocis megas* (Guénée) have been analyzed by gas chromatography and mass spectrometry (EI (electron impact) and CI (chemical ionization)). The female sex pheromone was a blend of (Z,Z,Z) 3,6,9 heneicosatriene (55%) and (Z,Z) 3,6-cis-9S,10R-epoxyheneicosadiene (45%). Male secretion produced at the level of a prothoracic organ was a blend of two unsaturated major hydrocarbons: (Z,Z) 6,9 heneicosadiene (64%) and (Z,Z,Z) 3,6,9 heneicosatriene (24%) and C_{19}, C_{20} and C_{22} homologues (total ratio 12%), as minor components. The trienic hydrocarbon was present in both sexes. The behavioral role of this male secretion has not yet been elucidated.
Key words. Sex pheromone; male secretion; chemical analysis; (Z,Z) 6,9 heneicosadiene; (Z,Z,Z) 3,6,9 heneicosatriene; (Z,Z)-3,6-cis-9S,10R-epoxyheneicosadiene; *Mocis megas*.

Noctuid moths of the genus *Mocis* (sub-family Catocalinae) are endemic in tropical areas. About 20 species are found from the southern United States to Brazil, including the Caribbean arc. *Mocis megas* (Guénée) was described for the first time from St. Thomas island, and is common from Puerto Rico to Dominica. The species has

no economic impact, although larvae can feed on various Graminaceae, including cultivated crops.

In the French Indies (Guadeloupe), *Mocis mega* is always to be found in damp areas on the edges of hygrophilic forests. This chemical analysis of the pheromone blends produced by males and females is part of a larger biosystematic project which aims at identifying chemotaxonomic characteristics of the species, and factors involved in the chemical isolation of *M. megas* from the sympatric species *Mocis latipes* (Guénée), which is harmful to fodder plants.

Female sex pheromones of Catocalinae have been identified for only four species: *Anticarsia gemmatalis* (Hubner)[1], *Caenurgina erechtea* (Cramer)[2], *Mocis latipes* (Guénée)[3] and *Mocis disseverans* (Walker)[4]. Male secretion has only been investigated in *A. gemmatalis*[5]. In all these cases, the compounds identified are homoconjugated long chain hydrocarbons.

Material and methods

Adults of *M. megas* emerged from eggs laid by five mated females caught using a light-trap near Petit-Bourg (Basse-Terre, Guadeloupe). Eggs were kept until emergence in cylindrical plastic boxes, provided with *Brachiaria purpurescens* stems. The caterpillars fed on the same plant under natural photoperiodic conditions (12 h light/12 h dark) at 25 °C. The total development cycle took about one month. Gas chromatographic analyses (GC) were performed on a Fractovap 2900 Carlo-Erba gas chromatograph equipped with a splitless injector and flame ionisation detector. Columns used were fused silica capillary columns: 25 m × 0.22 mm ID, Carbowax 57 CB, operating between 160 and 220 °C, temperature programmed at 2 °C/min; and 25 m × 0.22 mm ID, CP sil 8CB, operating between 45 and 160 °C, temperature programmed at 25 °C/min and between 160 and 220 °C at 2 °C/min. In both cases, helium was used as the carrier gas at a pressure of 1.9 bar for the first column and 0.9 bar for the second.

Mass spectra (MS) were produced using a Nermag R-10-10 instrument interfaced to a Girdel 30 GC equipped with a Ross injector. Electron impact MS were obtained at 70 eV. For chemical ionisation MS, nitrogen monoxide (NO) or ammonia (NH_3) was used as reagent gas.

Electroantennograms were recorded under standard conditions[6] (amount of test compound 0.5 µg, deposited on 1-cm^2 filter paper; stimulation time 0.5 s, air flow velocity 1.5–2 l/min).

Homoconjugated hydrocarbons were synthesized from their natural precursors (linoleic and linolenic acids) and obtained with a (Z) purity of 100%. Dienic epoxides were kindly donated by K. Mori[7] and J. R. Pougny[8] and were 98–99% optically pure.

Sex pheromone glands from 2-day-old virgin females were extracted at room temperature in nanograde hexane, during the maximum of the calling period (second half of the scotophase). Extracts (100 glands per 1 ml hexane) were concentrated under argon atmosphere to 100 µl and directly analyzed by GC and GC/MS.

The hair pencils of the males are localized on the upper part of the prothoracic pleura, near the parapatagia[9]. They were dissected from unmated 2-day-old males and extracted with the same solvent.

Results and discussion

GC analysis, on polar and non-polar capillary columns, of two hexane extracts from 77 and 91 sex pheromone glands of virgin females presented two main components (55 ± 3% of the first one; 45 ± 3% of the second) with retention times corresponding to a trienic homoconjugated C_{21} hydrocarbon and a related dienic epoxide, respectively.

Structure attributions were confirmed by mass spectrometry. The EI mass spectra of the first component had fragments at m/z: 234, 121, 108, 95, 93, 79 and 67 and a molecular ion at 290 (1%). The two fragments 234 (9%) and 108 (73%) were characteristic of a 3,6,9 heneicosatriene[3,10]. CI/NO mass spectra confirmed this hypothesis by enhancement of the ion intensity at 290 (58% molecular ion), 234 (100%) and 108 (98%) as previously reported[11].

The EI mass spectra of the second component did not produce any characteristic fragmentation (m/z: 122, 108, 93, 79 (base peak) and 67); whereas on CI/NO, a molecular ion appeared at 296 (1%), with its two parent ions at 336 and 305, and two other fragments at 183 ($C_{12}H_{23}O$) (91%) and 137 ($C_9H_{13}O$) (5%), characteristic of an oxirane ring located at 9,10 on a C_{21} straight chain[12]. All these data corresponded to a 9,10 epoxy 3,6 heneicosadiene. Geometry of the double bonds and of the epoxide ring have been assigned by comparison of its Kovats index with those of authentic samples on polar and non-polar GC capillary columns (table). We therefore conclude that the sex pheromone produced by virgin females of *Mocis megas* was a blend of (Z,Z,Z) 3,6,9 heneicosatriene (55 ± 3%) (**1**) and a derivative epoxide (Z,Z)-3,6 cis 9,10 epoxyheneicosadiene (45 ± 3%) (**2**) (fig. 1). It is the first time that this dienic epoxide has been identified in the sex pheromone blend of a noctuid moth, but it is known as the major pheromone component of many arctiid moths such as *Estigmene acrea*[13], *Hyphantria cunea*[14], *Creatonotos transiens* and *C. gangis*[15], *Tyria jacobaeae*[16] and *Cymbalophora pudica*[17].

To assign its absolute configuration, we recorded responses of the male antenna to the two authentic 9S,10R (−) and 9R, 10S (+) enantiomers, in comparison with 27 other compounds previously identified in Catocalinae, Arctiidae and Geometridae moths.

As shown in figure 2, the antenna produced a bimodal profile of EAG responses for homoconjugated C_{20} and C_{21} hydrocarbons, especially for those with (Z,Z,Z) 3,6,9 double bonds, and for the epoxides, mainly for the 9S,10R (−) enantiomer of (Z,Z)-3,6-cis-9,10 epoxyhene-

Kovats index, on non-polar (CPsil-5B) and polar (CPwax-57CB) capillary columns, of authentic samples and components isolated from sex pheromone gland extracts of females, and from thoracic hair pencil extracts of the males, of *Mocis megas* (Guénée).

	CPsil-5B		CPwax-57CB	
	Authentic samples K.I.	Extract K.I.	Authentic samples K.I.	Extract K.I.
C 16	1600	1600		
(Z,Z)-6,9-C_{21}	2068		2167	
(Z,Z,Z)-3,6,9-C_{21}	2074	2073	2232	2231
C 21	2100	2100	2100	2100
C 25			2500	2500
(Z)-6-cis-9,10-epoxy-C_{21}	2224		2523	
(E)-6-cis-9,10-epoxy-C_{21}	2224		2535	
(Z,Z)-3,6-cis-9,10-epoxy-C_{21}	2229	2229	2574	2575
(Z,E)-3,6-cis-9,10-epoxy-C_{21}	2244		2591	

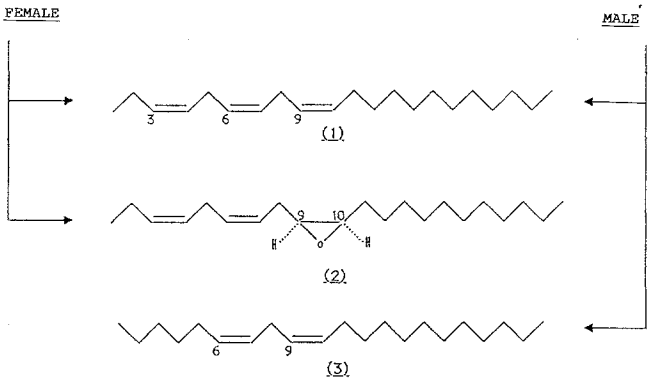

Figure 1. Chemical structures of the main pheromonal components identified from female sex pheromone gland and male thoracic hair pencils of *Mocis megas* (Guénée).

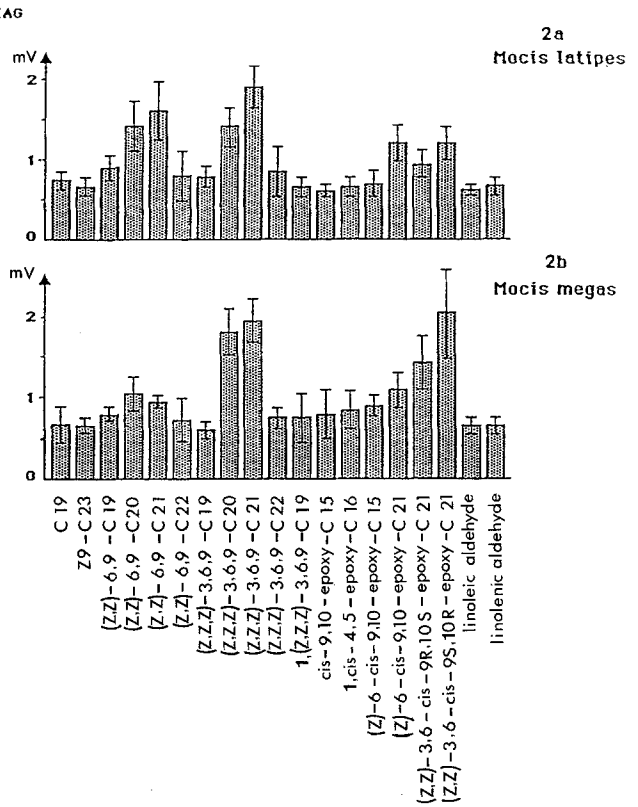

Figure 2. EAG profiles of responses of *Mocis latipes* males (2a) and *Mocis megas* males (2b) to various unsaturated hydrocarbons and related epoxides. Responses are corrected according to the average of responses to the entire series for each replicate (2–3 replicates per individual, 5 individuals tested).

icosadiene (key compound). This result suggested that the natural product had the same absolute configuration [16].

Under the same experimental conditions, the male antenna of the sympatric species *M. latipes* (fig. 2a) exhibited strong response only to homoconjugated hydrocarbons, mainly to (Z,Z,Z) 3,6,9 heneicosatriene (key compound) and (Z,Z) 3,6 heneicosadiene, which have been identified as the two components of its sex pheromone [3]. Thus (Z,Z)-3,6-cis-9S,10R epoxyheneicosadiene might be considered as a chemical factor resulting in the isolation of *M. latipes* from *M. megas*.

A hexane extract of hair pencils from 20 naive males was analyzed by GC and GC/MS. The GC trace showed two main components identified as before as the (Z,Z,Z) 3,6,9 heneicosatriene (**1**) (24%), and the (Z,Z) 6,9 heneicosadiene (**3**) (64%) (same mass spectra and Kovats index as those of authentic samples). Several minor compounds were also identified as related polyenic hydrocarbons: (Z,Z) 6,9 nonadecadiene (5%); (Z,Z,Z) 3,6,9 nonadecatriene (4%); (Z,Z) 6,9 eicosadiene (2%) and (Z,Z,Z) 3,6,9 eicosatriene (1%) with traces of (Z,Z) 6,9 docosadiene and (Z,Z,Z) 3,6,9 docosatriene.

Hair fringes present on the tibia of posterior legs of *M. megas* males were also extracted in a search for a possible other male secretion, as suggested by Barth in *Mocis repanda* [9]. But as no volatile could be detected either by GC or by GC/MS it seems that these hairs are not connected with secretory cells.

Conclusion

Until now, the chemical structures that have been identified in male secretions of noctuid moths have been found to be very different from those in the sex pheromones of conspecific females [18]. The great similarity observed here seems to be restricted to some genera belonging to the Catocalinae sub-family. But as only two species were investigated, a more comprehensive survey has to be undertaken to prove that this is a characteristic of Catocali-

nae, and to describe the behavioral significance of these compounds.

This is the first time that a long chain epoxide, such as (Z,Z)-3,6-cis-9S,10R-epoxyheneicosadiene, has been identified in a noctuid moth sex pheromone. Nevertheless, it has been found by EAG screening[19] that other neotropical Catocalinae males have either polyunsaturated hydrocarbons or epoxiderivatives as key compounds. The 9S, 10R enantiomeres always give the best EAG responses, except within the genus *Zale* where the opposite one; 9R,10S, is the key compound. In the field it has been found possible to attract males of *Zale duplicata* by (Z,Z)-3,6-cis-9R,10S epoxyheneicosadiene[20].

Great similarities exist between the sex pheromones of Catocalinae and Arctiidae moths. Pheromones identified in all the Arctiidae sub-families, including Ctenuchinae, are also blends of polyunsaturated hydrocarbons[10,21] or unsaturated epoxides[13,17], or mixtures of the two[15]. More experimental data are required before it can be concluded that there is a close relationship between Catocalinae and Arctiidae within the super-family Noctuoidea; in particular, studies of biochemical pathways of pheromone synthesis within Catocalinae that might be similar to that described for Arctiid moths[16,22].

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Gigantecin: A novel antimitotic and cytotoxic acetogenin, with nonadjacent tetrahydrofuran rings, from *Goniothalamus giganteus* (Annonaceae)

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Summary. Gigantecin (**I**), a novel tetrahydroxy-di-tetrahydrofuran fatty acid γ-lactone (acetogenin), was isolated from an ethanolic extract of the stem bark of *Goniothalamus giganteus* Hook. f., Thomas (Annonaceae), by means of activity-directed fractionation (brine shrimp lethality test). This new compound is extremely cytotoxic to human tumor cells, inhibits crown gall tumors on potato discs, and is active in an assay designed to detect antimitotic agents (9 ASK).
Key words. Gigantecin; acetogenins; *Goniothalamus giganteus*; Annonaceae; brine shrimp; antimitotic; cytotoxic; crown gall tumors; potato disc assay; 9 ASK.

Tetrahydrofuranoid acetogenins represent a new class[1] of bioactive compounds occurring in certain genera of the plant family Annonaceae. In recent years, our research has focused on the isolation and characterization of these diversely bioactive (antitumor, cytotoxic, pesticidal)[2-5] compounds. Extracts of the stem bark of *Goniothalamus giganteus* Hook. f., Thomas (Annonaceae) were pesticidal against four test organisms and exhibited significant murine toxicity in the 3 PS lymphocytic leukemia system[6] (toxic at 6.25 mg/kg). Our previous bioactivity-directed studies of the bark of *Goniothalamus giganteus* have yielded the bioactive compounds altholactone (syn.: goniothalenol, a furano-2-pyrone)[7], goniothalamin (a styrylpyrone)[7], and pinocembrin (5,7-dihydroxy