Alarm pheromones from the Mediterranean opisthobranch *Haminoea navicula*[1]

G. Cimino, A. Passeggio, G. Sodano[2], A. Spinella* and G. Villani

Istituto per la Chimica di Molecole di Interesse Biologico del C.N.R., Via Toiano 6, I-80072 Arco Felice, Napoli (Italy)
Received 2 April 1990; accepted 11 July 1990

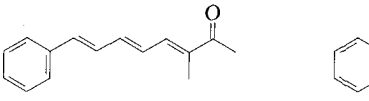
Summary. Structure and absolute stereochemistry of two new 3-alkylpyridines, haminol-A (**3**) and haminol-B (**4**), isolated from the cephalaspidean mollusc *Haminoea navicula*, have been determined by means of spectral and chemical methods. Haminols are secreted by *H. navicula* when it is molested, and these induce an alarm response in trail-following conspecifics.
Key words. Haminol-A, -B; marine alarm pheromones; *Haminoea navicula.*

Recent studies on Mediterranean cephalaspidean molluscs led to the characterization of a series of polypropionates, the aglajnes, from *Aglaja depicta*[3] and *Bulla striata*[4], and some ω-aryl conjugated methyl ketones, the lignarenones, from *Scaphander lignarius*[5]. The chemical structures of aglajnes suggested a predator-prey relationship between *A. depicta* and *B. striata*, while those of lignarenones, and in particular lignarenone-B (**1**), showed a surprising analogy with the structure of 3-methylnavenone-B (**2**), an alarm pheromone isolated[6] from the Pacific Algajidae *Navanax inermis.* Now we report the results of studies of another Mediterranean cephalaspidean mollusc, *Haminoea navicula*, belonging to a family (Haminoeidae) which suffers[8] predation by *A. depicta.*

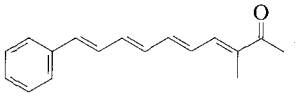
Haminoea navicula was collected in the Bay of Salerno at a depth of 4 m. 152 specimens, soaked in acetone, were subjected to ultrasonic treatment for 10 min. The diethyl ether soluble fraction (177 mg) from the acetone extract was fractionated on a silica gel column (light petroleum-diethyl ether 7:3) affording two compounds named in order of decreasing polarity: haminol-A (**3**, 22 mg) and haminol-B (**4**, 20 mg). Owing to their high instability, haminols have to be carefully protected from light during purification and storage.

The structure of haminol-A (**3**) was suggested on the basis of the following evidence: oil; $[\alpha]_D$ + 5° (c 0.3, CH_3OH); IR (liquid film) 3320 cm^{-1}; UV (CH_3OH) λ_{max}: 282 (ε = 4340), 232 (ε = 30200) nm; EIMS m/z (%): 257.1766 (M^+, 5, $C_{17}H_{23}NO$ requires 257.1780), 239 (M^+ − 18, 6), 212 (C_2–C_3 cleavage, 90), 132 (C_8–C_9 cleavage, 100); ^1H and ^{13}C-NMR assignments are reported in the table.

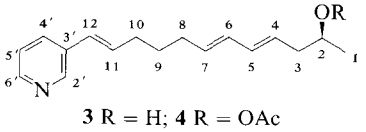
The ^1H-NMR spectrum was highly diagnostic for assigning the structure **3**. In fact, it showed four downfield signals (δ 8.54, 8.41, 7.64 and 7.21), easily attributable to the four protons of a 3-substituted pyridine ring. The comparison with the ^1H-NMR data[6,7] for navenone-A (**5**) further supported this assignment. Furthermore, the ^1H-NMR spectrum exhibited signals for two *trans*-oriented protons (δ 6.35, d, J = 16 Hz, H-12; δ 6.28, dt, J = 16 and 6.5 Hz, H-11) conjugated to the pyridine ring. The remaining two degrees of unsaturation were accounted for by four resonances (δ 5.57, 6.05, 6.10 and 5.62) assigned to the protons of a *trans-trans* conjugated diene. The spectrum also displayed signals easily assignable to a terminal $-CH_2CH(OH)CH_3$ system linked to the diene moiety. Finally, three multiplets (δ 2.13, 1.58 and 2.25) were assigned to the methylenes between C-7 and C-11. All the NMR assignments (table) were confirmed by both 2D NMR spectra (^1H-^1H-COSY and ^1H-^{13}C HECTOR) and ^1H-^1H decoupling experiments.



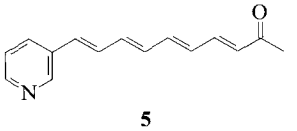
1



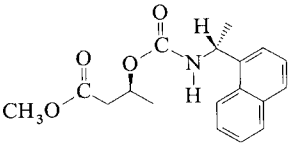
2



3 R = H; **4** R = OAc



5



6

The S absolute stereochemistry at C-2 was clarified by reaction of **3** with R(−)-1-(1-naphtyl)-ethyl-isocianate (benzene, 80 °C, 15 h) followed by a degradative Lemieux reaction ($KMnO_4$, KIO_4; pH8; 18 h) and methylation with CH_2N_2. The S configuration was assigned to the ester **6**, isolated from the reaction mixture, on the basis of the chemical shift value of the ester methyl (δ 3.58), following a previously established procedure[9].

The spectral data of **4** differed from those of **3** only in the presence of an acetoxy group at C-2. Haminol-B: oil; $[\alpha]_D$ − 24° (c 0.4, CH_3OH); IR (liquid film) 1730 cm^{-1}; UV (CH_3OH) λ_{max} 282 (ε = 3440), 230 (ε = 23400) nm; EIMS m/z (%): 299 (M^+, 5), 239 (90), 212 (10), 132

NMR data of haminols[a]

	Haminol-A (**3**)				Haminol-B (**4**)			
	δ $^{13}C^{b}$	m[c]	δ $^{1}H^{d}$	(m J Hz)	δ $^{13}C^{b}$	m[c]	δ $^{1}H^{d}$	(m J Hz)
1	22.7	q	1.20	(d 6.1)	19.4	q	1.21	(d 6.2)
2	67.3	d	3.83	(m)	70.4	d	4.92	(m)
3	42.5	t	2.25; 2.19	(m)	39.0	t	2.32; 2.26	(m)
4	127.8	d	5.57	(dt 14.7;7.4)	126.6	d	5.49	(dt 14.3;7.4)
5	130.6	d	6.05[e]	(dd 14.7;10.4)	130.6[f]	d	6.04	(m)
6	133.7	d	6.10[e]	(dd 14.7;10.4)	133.2[f,g]	d	6.04	(m)
7	132.9	d	5.62	(dt 14.7;7.1)	132.9	d	5.61	(m 14.3;7.2)
8	31.9	t	2.13	(m)	31.9	t	2.12	(m)
9	28.6	t	1.58	(m)	28.6	t	1.58	(m)
10	32.5	t	2.25	(m)	32.5	t	2.24	(m)
11	133.2	d	6.28	(dt 16;6.5)	133.3[f,g]	d	6.27	(dt 15.8;6.5)
12	126.7	d	6.35	(d 16)	126.6	d	6.35	(d 15.8)
2'	148.0	d	8.54	(bs)	148.0	d	8.54	(bs)
3'	133.5	s	–	–	133.4	s	–	–
4'	132.5	d	7.64	(bd 7.9)	132.4	d	7.64	(bd 7.9)
5'	123.4	d	7.21	(dd 7.9;4.5)	123.4	d	7.21	(dd 7.9;4.5)
6'	148.0	d	8.41	(bd 4.5)	148.0	d	8.42	(bd 4.5)
$\underline{C}OCH_3$					170.7	s	–	–
$CO\underline{C}H_3$					21.2	q	2.01	(s)

[a] WM 500 Bruker Spectrometer, $CDCl_3$; TMS = 0. [b] Assigned by ^{1}H-^{13}C HETCOR. [c] Deduced by DEPT sequence. [d] Assigned by ^{1}H-^{1}H COSY and ^{1}H-^{1}H decouplings. [e,f,g] Values with the same superscript could be interchanged.

(100); ^{1}H and ^{13}C-NMR are reported in the table. Acetylation of **3** yielded a compound identical to **4**, confirming the suggested structure.

Pyridine derivatives are very uncommon among marine metabolites[10]. However, they have been found in *Philinopsis speciosa*[11], which belongs to the same family as *N. inermis* and *A. depicta*, while, more recently, potent antileukemic 3-substituted pyridine metabolites have been found in the sponges *Niphates sp.*[12] and *Theonella swinhoei*[13].

Owing to the structural analogy of haminols with the alarm pheromones isolated from *N. inermis*[6,7,14], and in particular with navenone-A (**5**), haminols have been tested for their alarm pheromone properties. Field observations revealed that *H. navicula*, when molested, induces an alarm response in individuals following its trail (fig. 1). In aquarium bioassays (fig. 2), sand (50 mg) treated with a diethyl ether solution of haminol-A (or -B) was placed on the trail of a specimen of *H. navicula* using a Pasteur pipette (fig. 2b). It was observed that the next individual following the trail, when in contact with the treated sand, rapidly deviated and traced a second trail (fig. 2c,d). The alarm response was induced at a concentration of 0.3 mg for **3** and of 0.1 mg for **4**. No deviation was observed using sand treated only with the solvent.

Haminols can be added to the list of the few marine alarm pheromones whose structures have been described. In fact, even though several examples of intraspecific chemical communication are known in the marine environment, only seldom has the chemistry of these pheromones been defined[7].

Haminoea navicula, Scaphander lignarius and *Navanax inermis* are all cephalaspidean opisthobranchs. It is apparent at the present time that members of different genera are capable of the production of navenone-like compounds. However, it should be taken into account that

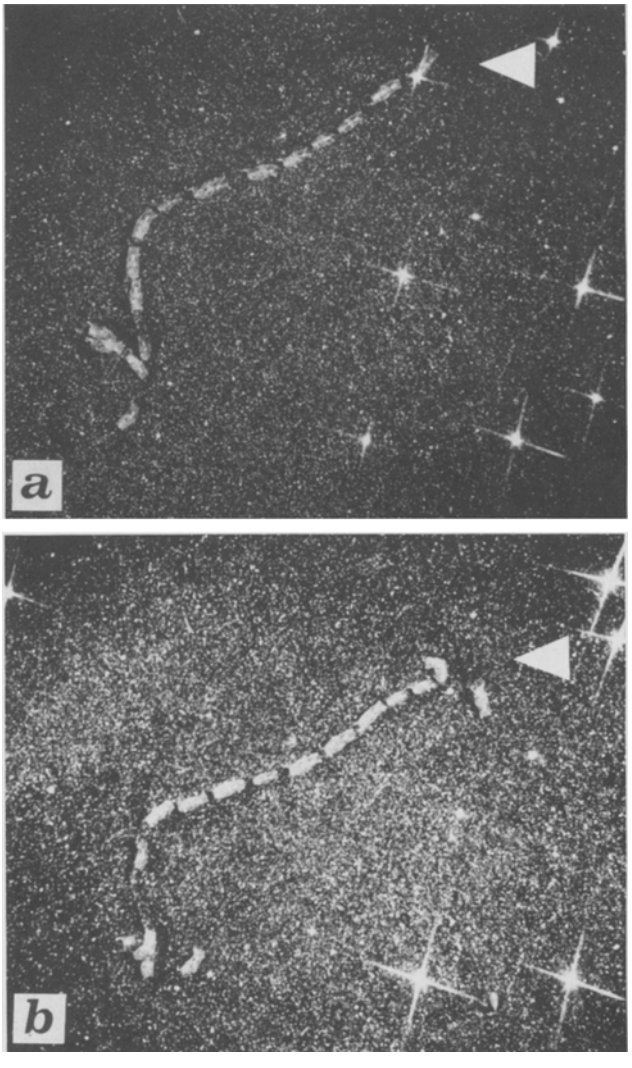


Figure 1. Field observation of alarm response: (*a*) typical line of *H. navicula* specimens; (*b*) after the first mollusc (arrowed) had been molested, a change in direction was observed for the trail-following specimen.

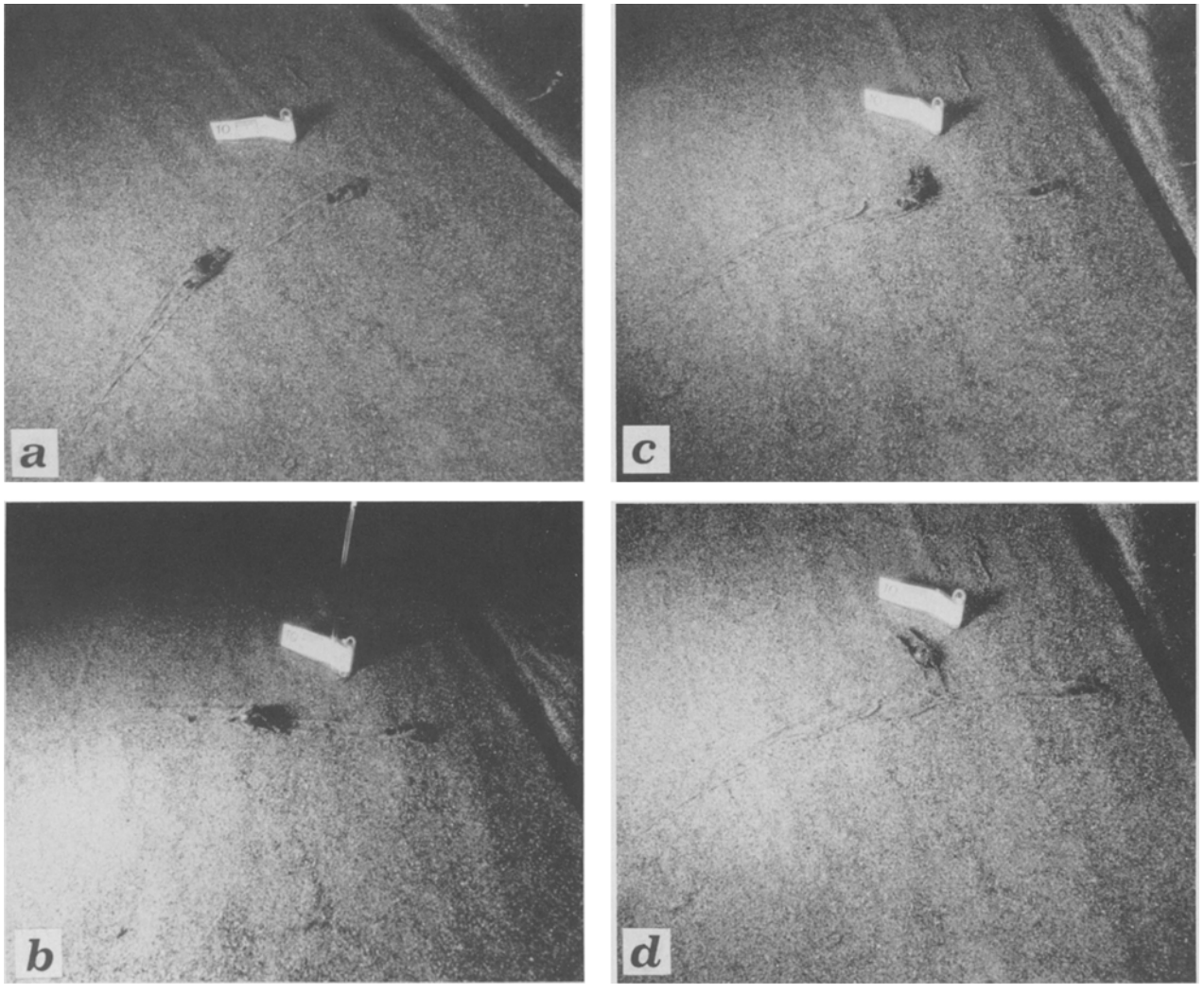


Figure 2. Alarm response of *H. navicula* observed in the laboratory: (*a*) typical trail-following behavior; (*b*) introduction of haminol-A on the trail by Pasteur pipette; (*c,d*) deviation observed.

the larger *N. inermis* is a very voracious mollusc which eats other opisthobranchs[15], in particular *Bulla gouldiana* and *Haminoea virescens*[7], and therefore the possibility cannot be excluded that the navenones in *N. inermis* could have a dietary origin from the prey. In this respect, it is interesting to report that when the Mediterranean *A. depicta*, usually a predator of *B. striata*[4,8], was induced in the aquarium to prey on *H. navicula*, haminols were found both in the digestive glands of *A. depicta* and in its yellow secretions.

Acknowledgments. The authors are grateful to Prof. W. Fenical (University of California, San Diego) for fruitful discussions. Thanks are also extended to Mr A. Crispino and Mr A. Trabucco for the collection of the molluscs, to Mr R. Turco for the graphic assistance and to Mrs M. R. Vaccaro for the secretarial help. The NMR spectra have been recorded at the ICMIB-NMR Service. The mass spectra were provided by the 'Servizio di Spettrometria di Massa'. Both the staffs are gratefully acknowledged.

* To whom correspondence should be addressed.

1 In part presented at the 32nd IUPAC Congress Stockholm, 2–7 August 1989. Cimino, G., and Sodano, G., Abstracts SL 302, p. 63.

2 Current address: Dipartimento di Fisica, Università di Salerno, I-84081 Baronissi (SA), Italy.
3 Cimino, G., Sodano, G., Spinella, A., and Trivellone, E., Tetrahedron Lett. *26* (1985) 3389.
4 Cimino, G., Sodano, G., and Spinella, A., J. org. Chem. *52* (1987) 5326.
5 Cimino, G., Spinella, A., and Sodano, G., Tetrahedron Lett. *30* (1989) 5003.
6 Sleeper, H. L., and Fenical, W., J. Am. chem. Soc. *99* (1977) 2367.
7 Sleeper, H. L., Paul, V. J., and Fenical, W. S., J. chem. Ecol. *6* (1980) 57 and references therein.
8 Fasulo, G., Izzillo, F., and Villani, G., Boll. Malacologico *18* (1982) 97.
9 Cimino, G., Spinella, A., Scopa, A., and Sodano, G., Tetrahedron Lett. *30* (1989) 1147.
10 Faulkner, D. J., Natl Prod. Rep. *5* (1988) 613 and previous reports.
11 Coval, S. J., and Scheuer, P. J., J. org. Chem. *50* (1985) 3024.
12 Quinoa, E., and Crews, P., Tetrahedron Lett. *28* (1987) 2467.
13 Kobayashi, J., Murayama, T., Ohizumi, Y., Sasaki, T., Ohta, T., and Nozoe, S., Tetrahedron Lett. *30* (1989) 4833.
14 Fenical, W., Sleeper, H. L., Paul, V. J., Stallard, U. O., and Sun, H. H., Pure appl. Chem. *51* (1979) 1865.
15 Paine, R. T., Veliger *6* (1963) 1.

0014-4754/91/010061-03$1.50 + 0.20/0