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Alkylphenols from the Cephalaspidean Mollusc *Haminoea callidegenita*

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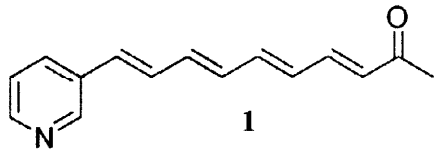
Abstract:. Alkylphenols **6-10** are the main metabolites isolated from the parapodia extract of the marine mollusc *Haminoea callidegenita*. The structure and the anatomical location of these compounds strongly support their potential defensive role as alarm pheromones.

Some years ago a series of exciting papers reported the chemical characterization, the biosynthesis and the biological properties of some interesting molecules, navenones A (**1**), B (**2**) and C (**3**), found in the cephalaspidean *Navanax inermis* and possessing alarm pheromone activity.[4,5] The pheromones were released by the mollusc in the mucus trail and this induced alarm response in a following conspecific specimen.[6] Further studies of the metabolites from *Navanax inermis* were not able to detect these very unstable compounds but led to some dietary polypropionates obtained from the mollusc *Bulla gouldiana*.[7]



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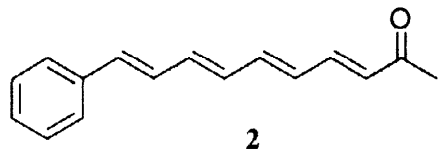

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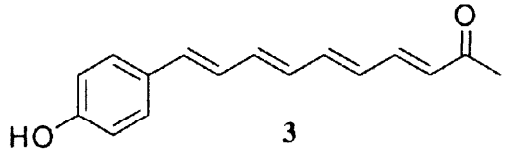

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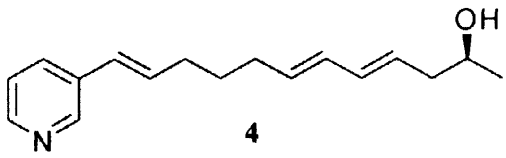

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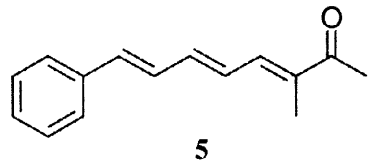

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It is interesting that molecules related to navenones A (**1**) and B(**2**) have been found in other cephalaspideans, e. g. **4** [8] and **5**,[9] whereas until now linear molecules with a terminal phenol, like navenone C (**3**), have not been yet isolated from other molluscs. In this paper, we report the chemical study of *Haminoea callidegenita* which contains phenols, and their acetate derivatives, with a ten carbon alkyl chain bearing an ω-acetoxy substituent.

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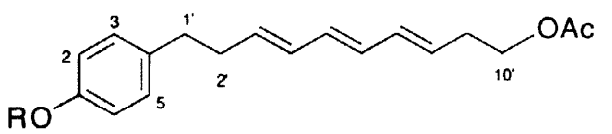
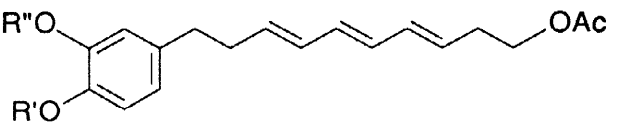
Haminoea callidegenita is a cephalaspidean mollusc which was firstly described from the Pacific coasts [10] and during this work it was collected for the first time from both Mediterranean (Giudecca, Venezia, Italy; May 1992) and Atlantic (Asturias, North of Spain; February 1993) coasts.

Mediterranean *H. callidegenita* (195 specimens) was frozen and then, soaked in acetone, subjected to sonication. The diethyl ether soluble fraction of the acetone extract (240 mg) was chromatographed on silica gel using ligth petroleum ether with increasing content of diethyl ether (from 20% up to 100%). Several purifications allowed to obtain five fractions containing, in order of increasing polarity, respectively compounds **6** (4.9 mg), **7** (3 mg), **8** (4.3 mg), a mixture of compounds **9** and **10** (2.4 mg) and, furthermore, some polypropionate compounds whose structures have to be determined.

Compound **6** is an oil, the MS spectrum showed a strong peak at *m/z* 268.1461 according to the elemental composition $C_{18}H_{20}O_2$ (cld 268.146321) but the NMR analysis suggested that the peak at 268 is a fragment deriving from the molecular peak by loss of acetic acid. In fact, the ^{1}H-NMR spectrum, showing an AB system at δ 6.99 and 7.18, suggested the presence of a *para* disubstituted aromatic ring. The presence of an acetoxy substituent in this ring was suggested by the IR band at 1759 cm^{-1}, together with the methyl resonance at 2.29 δ and the highfield shift of the two *ortho* aromatic protons. Starting from the two protons resonating at δ 2.70 (H_2-1') the nature of the alkyl chain was rapidly clarified. In fact, the protons at δ 2.40 (H_2-2') were coupled both with H_2-1' and with the first olefinic proton (H-3') of a triene system substituted at the other end by a methylene coupled with another methylene bearing an acetoxy group.



6 R=Ac
7 R=H



8 R'=Ac ; R"=Ac
9 R'=Ac ; R"=H
10 R'=H ; R"=Ac

The ^{13}C-NMR data (Table) well agree with the proposed structure. The stereochemistry of the conjugated triene is suggested all-*trans* even though direct evidence supported only the *trans* configuration of $\Delta^{3'}$ ($J_{H3',H4'}$= 14.6 Hz) and $\Delta^{7'}$ ($J_{H7',H8'}$= 14.9 Hz) . However, the ^{13}C-NMR value of all the alkene carbons should exclude a *cis* configuration at $\Delta^{5'}$.[11]

The mass spectrum of **7** showed the molecular peak at *m/z* 286 and an intense fragment at *m/z* 226 (M^+ - 60), whereas the IR spectrum exhibited only a carbonyl band (1735 cm^{-1}) and a wide band centered at 3430 cm^{-1}. All NMR data of **7** (Table), and in particular the absence of the methyl signal at δ 2.29, well fit with a structure closely related to **6**, but deacetylated at C-1. Acetylation of **7** confirmed the suggested chemical correlation.

The data of compound **8** were closely related to those of **6** and **7**, the alkyl chain was substantially the same, whereas another acetoxy substituent was placed on the aromatic ring. In fact, only three aromatic protons were present along with three acetoxy methyls (at δ 2.28, 2.27 and δ 2.04). Two aromatic protons showed an *ortho* coupling whereas the third was *para* coupled with one of them. The new acetoxy group was placed at position 2 on the basis of general NMR rules. In particular the ^{13}C-NMR values of C-6 (δ =123.1) and C-3 (δ =123.2) shielded by the adjacent acetoxy group were diagnostic. In fact, calculation of ^{13}C NMR chemical shifts

for trisubstituted aromatic rings with one alkyl and two acetoxy groups showed that the best fitting with the experimental data were those corresponding to substituents in 1, 2, 4 positions as in **8**.[12]

The last fraction showed a pattern of signals similar to that of compound **8**. However, the intensity of the acetoxy methyl signals, at δ 2.04 and 2.35, was according with the presence of only two acetoxy groups. Furthermore, the wide IR band centered at 3432 cm^{-1} supported the presence of an OH functionality. Therefore it was clear that starting from structure **8** an acetoxy group has to be removed. In fact, acetylation of this fraction gave compound **8**. In order to understand which acetoxy group was missing this fraction was subjected to methylation with diazomethane. This reaction yielded a mixture (1:1) of two methoxy acetoxy derivatives (methoxy groups at δ 3.85 and 3.86) showing that this fraction was really a mixture of monoacetoxy compounds **9** and **10**.[13]

Table: NMR Data [a] for Compounds **6**, **7** and **8**.

	6		**7**	**8**	
	δ ^{1}H (m, *J* Hz)	δ ^{13}C (m)	δ ^{1}H (m, *J* Hz)	δ ^{1}H (m, *J* Hz)	δ ^{13}C (m)
1	-	148.8 (s)	-	-	140.3[c](s)
2	6.99 (d, 8.6)	121.3 (d)	6.74 (d, 8.4)	-	142.1[c](s)
3	7.18 (d, 8.6)	129.3 (d)	7.04 (d, 8.4)	6.99 (d, 1.8)	123.2 (d)
4	-	139.3 (s)	-	-	140.0[c](s)
5	7.18 (d, 8.6)	129.3 (d)	7.04 (d, 8.4)	7.05 (dd, 8.2, 1.8)	126.5 (d)
6	6.99 (d, 8.6)	121.3 (d)	6.74 (d, 8.4)	7.09 (d, 8.2)	123.1 (d)
1'	2.70 (t, 7.5)	35.1 (t)	2.63 (t, 7.3)	2.70 (t, 7.5)	35.1 (t)
2'	2.40 (dt, 7.1, 7.5)	34.5 (t)	2.37 (dt, 7.1, 7.3)	2.41 (dt, 7.1, 7.5)	34.2 (t)
3'	5.71 (dt,14.6, 7.1)	133.7 (d)	5.70 (dt, 15.0, 7.1)	5.70 (dt, 14.3, 7.1)	133.3 (d)
4'	6.14 - 6.04	130.7[b](d)	6.13 - 6.03	6.12 - 6.06	130.9[d](d)
5'		131.0[b](d)			131.1[d](d)
6'		131.8[b](d)			131.7[d](d)
7'		132.9[b](d)			132.9[d](d)
8'	5.61 (dt, 14.9, 7.1)	128.7 (d)	5.60 (dt, 15.0, 7.1)	5.61 (dt, 14.2, 7.1)	128.8 (d)
9'	2.42 (dt, 6.8, 7.1)	32.1 (t)	2.42 (dt, 6.8, 7.1)	2.43 (dt, 6.8, 7.1)	32.1 (t)
10'	4.10 (t, 6.8)	63.7 (t)	4.10 (t, 6.8)	4.10 (t, 6.8)	63.7 (t)
$COCH_3$	2.29 (s)	21.1 (q)	2.04	2.28 (s)	20.6 (q)
$COCH_3$	-	169.6 (s)	-	-	168.6 (s)
$COCH_3$	2.04 (s)	21.0 (q)		2.27 (s)	20.6 (q)
$COCH_3$	-	169.6 (s)		-	168.6 (s)
$COCH_3$				2.04 (s)	21.0 (q)
$COCH_3$				-	171.3 (s)

[a] Bruker 500 AMX; $CDCl_3$; chemical shifts referred to $CHCl_3$ at 7.26 ppm and to $CDCl_3$ at 77.0 ppm. (Assignments aided by two-dimensional experiments: COSY, HMQC, HMBC). [b,c,d] Values with the same superscript could be interchanged.

Careful dissection of these small animals showed that these compounds (**6**-**10**) are exclusively located on the external part of the mollusc (parapodia) while they are completely absent in the internal organs. This finding is in agreement with their potential alarm pheromone activity. In fact, this compartmentalization allows the release of the alkylphenols in the mucus trail generally observed when *Haminoea* moves.

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13. **9**, **10** mixture: ^{1}H-NMR ($CDCl_3$, 500 MHz) 6.99 (d; 8.2 Hz), 6.92 (m), 6.83 (d; 2.1 Hz), 6.73 (dd; 2.1, 8.2 Hz), 6.12-6.09 (m; 4H), 5.71 (m), 5.61 (m), 4.10 (t; 6.8 Hz), 2.64 (m), 2.42 (m), 2.41 (m), 2.35 (s), 2.04 (s).