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POTENTIAL ALARM PHEROMONES FROM THE MEDITERRANEAN OPISTHOBRANCH *SCAPHANDER LIGNARIUS*

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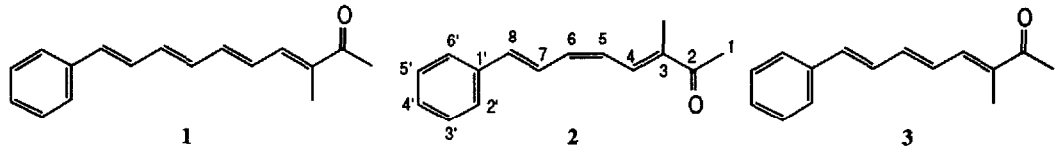
Abstract.- Two new ω-phenyl conjugated trienones, lignarenone-A (**2**) and lignarenone-B (**3**), are the main metabolites isolated from the dorsum acetone extract of *Scaphander lignarius*. Their structures, closely related to 3-methyl navenone-B (**1**) a minor component of the alarm pheromone mixture of the opisthobranch *Navanax inermis*, were characterized by spectral methods.

Defense allomones isolated from opisthobranch molluscs[1] fall mainly into two categories, ichthyotoxins and antifeedants. However, molecules acting as alarm pheromones have been reported in the Pacific opisthobranch *Navanax inermis*[2-4] (Bullomorpha, Aglajidae) which, when molested, secretes in its own slime trail some pheromones, navenones A-C and related minor compounds, which induce an alarm response in the trail following *Navanax*. In this paper we report the isolation from the Mediterranean bullomorph *Scaphander lignarius* (Scaphandridae[6]) of two compounds closely related to 3-methyl navenone-B (**1**)[4,5].



1 **2** **3**

300 specimens of *S. lignarius* were frozen, deshelled and dissected keeping mantles and digestive glands apart. Silica gel column chromatography of the diethyl ether solubles of an acetone extract of the mantles yielded in order of increasing polarity lignarenone-A (**2**, 65 mg) and lignarenone-B (**3**, 55 mg). The structures were assigned on the basis of the following evidence.

Lignarenone-A (**2**), $C_{15}H_{16}O$ by HREIMS (obs. 212.1208; calc. 212.1201), shows the following spectral data. EIMS, *m/z* (%) 212 (M^+, 67), 197 (18), 169 (loss of the terminal methyl ketone group, 20), 91 (100). IR (liquid film) ν_{max} 1660 cm^{-1}. UV (CH_3OH) λ_{max} (ε) 350 (42,606), 286 (16,090), 262 (13,590) nm. A combination of homonuclear and heteronuclear two dimensional methods allowed the assignment of all the ^1H and ^{13}C NMR chemical shifts (Table). The vinylic methyl was placed at C-3, analogously to **1**, on the basis of the ^1H NMR chemical shift values of H-4 (δ 7.61) and H-8 (δ 6.73)[4]. Furthermore, ozonolysis of **2** (CH_2Cl_2, -78°C), followed by reaction with *o*-phenylendiamine

(py, r.t.), led to the identification in the reaction mixture of 2,3-dimethylquinoxaline (^{1}H NMR, δ 2.75, s, 6H's) by comparison with an anthentic sample. The stereochemistry of the 5,6 and 7,8 double bonds was easily assigned by analysis of the coupling constant values of the olefinic protons, while the stereochemistry of the 3,4 double bond was assigned on the basis of the ^{13}C resonance of the vinylic methyl (δ 11.4).

TABLE - NMR Data of lignarenones[a]

	Lignarenone-A (**5**)				Lignarenone-B (**6**)			
C	δ ^{13}C	m[b]	δ ^{1}H[c]	(m; J in Hz)	δ ^{13}C	m[b]	δ ^{1}H[c]	(m; J in Hz)
1	25.8	d	2.42	(s)	25.4	q	2.35	(s)
2	199.2	s	-		198.9	s	-	
3	136.7[d]	s	-		136.8[e]	s	-	
4	132.7	d	7.61	(d; 11.8)	138.7	d	7.10	(d; 10.3)
5	124.6	d	6.38	(bt; 11.4)	128.6	d	6.67	(dd; 15.0, 10.3)
6	136.1	d	6.50	(bt; 11.2)	136.1[f]	d	6.72	(dd; 15.0, 9.6)
7	123.3	d	7.31	(dd; 15.4, 11.2)	128.5	d	6.92	(dd; 15.4, 9.6)
8	136.9	d	6.73	(d; 15.4)	139.8[f]	d	6.71	(d; 15.4)
CH_3-3	11.4	q	1.93	(s)	11.6	q	1.93	(s)
1'	136.8[d]	s	-		136.4[e]	s	-	
2'-6'	126.8	d	7.47	(d; 7.4)	126.7	d	7.43	(bd; 7.8)
3'-5'	128.7	d	7.34	(m)	128.7	d	7.33	(m)
4'	126.1	d	7.28	(m)	128.2	d	7.25	(m)

a WM 500 Bruker Spectrometer; $CDCl_3$; TMS=0.

b Deduced by DEPT sequence.

c Assigned by analysis of homo- and heteronuclear 2D experiments.

d,e,f Values with the same superscript could be interchanged.

The spectral data of lignarenone-B (**3**) suggested a close relationship with **2**. EIMS m/z (%) 212 (M^+, 100), 197 (40), 169 (60). IR (liquid film) ν_{max} 1657 cm^{-1}. UV (CH_3OH) λ_{max} (ε) 350 (85,106), 255 (6,090), 246 (10,178), 238 (7,686) nm. The all-*trans* geometry for the conjugated triene was deduced from the NMR data (Table).

Bearing in mind the biological properties of navenones[4], it is highly probable that lignarenones also possess pheromone properties. However, it has been impossible to confirm this hypothesis with adequate experiments as the animals, collected by dredging at a depth of 80-100 m, were obtained highly stressed and survived only for few hours. Studies are in progress on the alarm pheromones of other bullomorphs (*Akera bullata*, *Haminoea navicula*, *Haminoea hydatis*).

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