First Chemical Study of Patagonian Nudibranchs: A New Seco-11,12-spongiane, Tyrinnal, from the Defensive Organs of *Tyrinna nobilis*

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The Patagonian nudibranch *Tyrinna nobilis* contains a number of terpenoids, the novel seco-11,12-spongiane tyrinnal (**1**) and the known sesquiterpenoids dendrolasin (**2**), pallescensin A (**3**), and dehydropallescensin-2 (**4**). The metabolites probably derive from dietary sponges, thus suggesting a parallelism between the ecological relationships of *T. nobilis* and those of mollusks of genus *Cadlina*. The structure of **1** was determined by spectroscopic methods.

Mollusks of the family Chromodorididae show an interesting defensive strategy mainly based on chemicals.[2−4] These substances, always related to sponge metabolites, are stored in specific body areas or expelled with mucus.[5] It is generally accepted that the animals are able to intake their defensive allomones from their prey, and, recently, we have demonstrated this aptitude in *Hypselodoris webbi* by experiments in an aquarium.[6] In this paper, we report the first ecological survey of a Patagonian mollusk, the dorid *Tyrinna nobilis* Bergh, 1898 (Opisthobranchia: Nudibranchia: Chromodorididae) and the novel structure of the presumed defensive allomone tyrinnal (**1**) isolated from its skin.

The nudibranch *T. nobilis* (10 specimens) was collected off the Patagonian coast in December 1995. Optical microscope revealed small round structures along the animal body reminiscent of the mantle dermal formations (MDFs) already described in Mediterranean *Hypselodoris* nudibranchs.[7] Following our standard procedures,[5,6] the frozen animals were dissected, and the extracts from skin organs (MDFs), mantle, inner components, and mucus were prepared. TLC analysis of these fractions showed several products positive to Ehrlich's reagent and revealed their compartmentalization in the extracts from mucus and MDFs. Si gel chromatography of the combined extracts gave dendrolasin (**2**) and a mixture of other two furanosesquiterpenoids resolved into pallescensin A (**3**) and dehydropallescensin-2 (**4**) by further column chromatography on 4% $AgNO_3-SiO_2$. In addition, reversed-phase HPLC (MeOH−H_2O 80:20) of more polar components yielded **1** (0.8 mg) together with minor uncharacterized compounds.

Tyrinnal (**1**), $[\alpha]^{25}_D$ −14.7° (*c* 0.3, $CHCl_3$), had the molecular formula $C_{24}H_{36}O_6$, with the highest peak in its EIMS occurring at *m/z* 360 corresponding to the loss of HOAc from the parent molecule. The ^1H NMR spectrum in $CDCl_3$ exhibited signals for two acetyl (*δ* 2.10 and 2.12) and four angular methyl groups (CH_3-17, *δ* 0.84; CH_3-18, *δ* 0.87; CH_3-19, *δ* 1.24; CH_3-20, *δ* 1.39). The downfield region included two methine hydrogens at *δ* 6.58 (H-15) and *δ* 6.50 (H-16), an AB system centered at *δ* 4.78 and 4.61 (H_2-12), and a doublet (J = 4.3 Hz) for an aldehyde function at *δ* 9.94. These data were appropriate for a rearranged spongiane skeleton, although overlapping signals did not allow the complete characterization of the molecule. The spectrum of **1**, however, was satisfactorily resolved in C_6D_6, where decoupling experiments revealed a strong correlation between the aldehyde signal (*δ* 9.83) and a sharp doublet at *δ* 2.15 (H-9). The 1-acetoxy-1,2-dihydrofuran ring was identified by the carbon resonances at *δ* 147.3 (C-16), 111.7 (C-13), 100.1 (C-15), and 59.8 (C-14), with the hemiacetal proton at *δ* 7.08 coupled to the methine hydrogen at *δ* 3.02 (H-14). On the other hand, the olefin H-16 (*δ* 6.24) showed weak, but very diagnostic couplings with the hydroxymethylene signals at *δ* 4.69 and 4.42 (J = 13.0 Hz, H_2-12). HMQC and COSY analysis revealed the presence of an isolated $CHCH_2CH_2$ spin system (H-5, *δ* 0.61; H_2-6, *δ* 1.18 and 1.35; H_2-7, *δ* 1.27) and also allowed identification of all resonances in ring A (Table 1). Upon examination of NOE difference spectra, irradiation of the H-9 signal produced a strong enhancement (19%) of H-15 (*δ* 7.08). This effect was due to one of the energetically favored conformers of **1** in which H-9 and H-15 come near because of a 180° rotation of the furan moiety around the C-8−C-14 bond (Figure 1). Significant through-space interactions (Figure 1) were also found between CH_3-17 (*δ* 0.68) and CH_3-19 (*δ* 0.96), as well as between

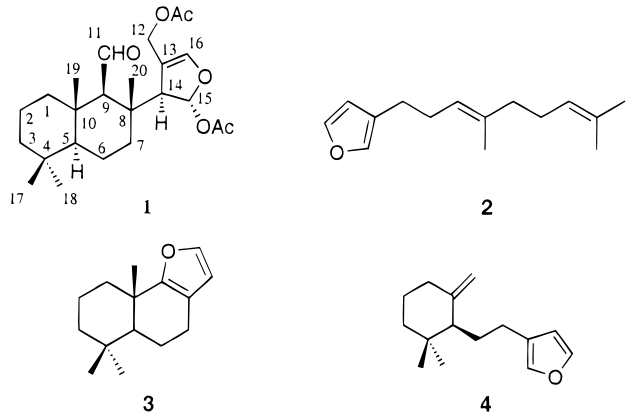

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Table 1. NMR Data of Tyrinnal (**1**)[a]

	$CDCl_3$		C_6D_6			
no.	^1H	^{13}C	^1H	^{13}C	HMBC	NOEs
1	1.62 m	40.8 (t)	1.4 m	40.8 (t)		
	1.16 m		1.04 m			
2	1.45 m	17.9 (t)	1.35 m	18.4 (t)		
	1.18 m					
3	1.47 m	41.4 (t)	0.97 m	41.4 (t)		
	1.21 m		1.18 m			
4		34.3 (s)		33.7 (s)		
5	0.83 d 10.9 Hz	55.1 (d)	0.61 brd 12.0 Hz	55.5 (d)		
6	1.63 m	18.1 (t)	1.18 m	18.2 (t)		
	1.47 m		1.35 m			
7	1.40 m	36.2 (t)	1.27 m	36.4 (t)		
	1.26 m					
8				39.9 (q)		
9	2.03 d 3.9 Hz	66.0 (d)	2.15 d 4.0 Hz	66.4 (d)	C-10,C-11,C-20	H-15
10		39.6 (s)		39.6 (s)		
11	9.94 d 4.3 Hz	206.6 (d)	9.83 d 4.0 Hz	205.4 (d)		Me-19,Me-20
12	4.78 d 13.0 Hz	59.8 (t)	4.6 d 13.0 Hz	60.0 (t)	C-16	
	4.69 d 13.0 Hz		4.42 d 13.0 Hz			
13				111.7 (s)		
14	2.81 brs	59.2 (d)	3.02 brs	59.8 (d)	C-15	Me-20
15	6.58 brd 2.1 Hz	99.6 (d)	7.08 brd 0.4 Hz	100.1 (d)	C-13,C-14	H-9,Me-20
16	6.50 s	146.7 (d)	6.24 brs	147.3 (d)	C-13	
17	0.84 s	21.0 (q)	0.68 s	21.7 (q)	C-5,C-4	Me-19
18	0.87 3	33.0 (q)	0.68 3	33.1 (q)	C-5,C-4	
19	1.24 s	17.9 (q)	0.96 s	17.8 (q)	C-10,C-5	H-11,Me-17,Me-20
20	1.39 s	21.5 (q)	1.21 s	21.7 (q)	C-7, C-9, C-14	H-11,H-14,Me-20
CH_3	2.10 s	20.8 (q)	1.72 s	21.7 (q)		
CH_3	2.12 s	20.8 (q)	1.77 *s*	21.7 (q)		
CO		170.1 (q)		171.4 (q)		
CO		170.1 (q)		169.7 (q)		

[a] All experiments were performed at 500 MHz. *J* values are reported in Hertz, and chemical shifts are given in δ units referenced to $CHCl_3$ (7.26 and 77.0 ppm) and C_6D_5H (7.14 and 128.0 ppm).

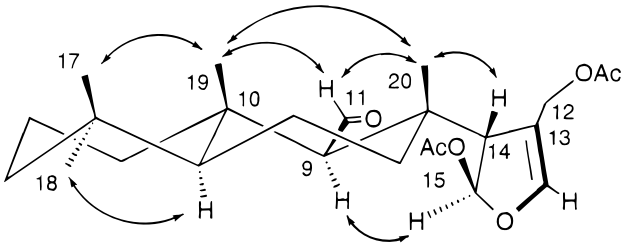

Figure 1. Stereochemical view of tyrinnal (**1**) and diagnostic NOEs.

H-11 (δ 9.83), CH_3-19 (δ 0.96), and CH_3-20 (δ 1.21), proving the cis relation of these groups as expected for a spongiane derivative. Moreover, the presence of intense NOEs both between H-9 and H-15 and between H-14 and CH_3-20 suggested a trans relation of H-14 and H-15. Finally, HMBC data confirmed the above assignments, providing heteronuclear couplings between the quaternary carbons C-4, C-10 and C-13 with the adjacent hydrogens (Table 1). Compounds **2**−**4**[8−10] were identified by comparing their spectral data (^1H NMR, $[\alpha]_D$) with those reported in the literature.

Spongiane compounds have been isolated from nudibranchs[11,12] and sponges of genus *Spongia*.[13] At the moment, we cannot prove whether *Tyrinna* is able to transform a sponge precursor or whether the mollusk simply sequesters and accumulates a preexisting sponge metabolite. Among nudibranchs of the family Chromodorididae, *T. nobilis* shares some analogies with *Cadlina* species.[14] Although the presence of MDFs in *T. nobilis* represents a basic anatomical difference, the skin extracts of both animals are featured by furanosesquiterpene and spongiane compounds. Assuming the dietary origin of these metabolites, this suggests that the mollusks eat different sponges and that they do not have a specialized diet as other chromodoridids (*Hypselodoris*, *Chromodoris*).[14] We could not demonstrate conclusively the defensive role of tyrinnal (**1**), but such a function is strongly favored from biological and ecochemical arguments.

Experimental Section

General Methods. 1D and 2D NMR spectra were recorded on Bruker AMX-500. The $CHCl_3$ resonances at δ 7.26 and 77.0 were used as internal references. MS were obtained on a Kratos MS 50 spectrometer operating at 70 eV. IR data were recorded by a BIO-RAD FTS-7 FT/IR spectrophotometer. Optical rotations were determined by a JASCO DIP-370 polarimeter. HPLC was performed by Waters liquid chromatography apparatus equipped with two 510 pump units and a JASCO Uvidec 100 III spectrophotometer.

Collection, Extraction, and Purification. The nudibranchs (10 specimens) were collected off Patagonian coasts (Punta Pardelas, Punta Marquez, and Punta Maqueda, Argentina).[15] A voucher specimen is kept at the Museo Argentino de Ciencias Naturales "Bernardino Ridavia" (voucher nos. MACN: 33872, 33873, and 33874). The frozen animals were dissected under optical microscope into viscera, mantle, and MDFs and extracted with Me_2C. After removing the volatile solvent, the residues were diluted by fresh H_2O and separately partitioned with Et_2O. The organic layers were dried on Na_2SO_4, filtered on paper, and evaporated to small volume. After TLC comparison, the extracts of MDFs and mantle were combined (212 mg) and

fractionated on Si gel to give in the earlier fractions dendrolasin (**2**, 4.3 mg) and a mixture (11.8 mg) of pallescensin A (**3**) and dehydropallescensin-2 (**4**), which were further separated by $AgNO_3-SiO_2$ column. On the other hand, fractions 5 and 6 (24 mg), which showed products positive to Ehrlich's reagent, were chromatographed by reversed-phase HPLC (Spherisorb ODS-2 column, 10 × 250 mm, MeOH−H_2O 80:20, 3 mL/min, detector UV 205 nm) to afford **1** (0.8 mg, t_R 31 min) and other five peaks containing uncharacterized Ehrlich postive compounds.

Tyrinnal (1): colorless oil (0.8 mg), $[\alpha]^{25}_D$ −14.7° (*c* 0.3, $CHCl_3$); IR (film) ν_{max} 2924, 2367, 1742, 1457, 1227, 1039 cm^{-1}; NMR data, see Table 1; EIMS (*m*/*z*) 360 (20), 300 (42), 285 (35), 219 (35), 123 (90), 97 (100); HREIMS *m*/*z* 360.2314 [calcd for $C_{22}H_{32}O_4$, $(M-60)^+$, 360.2328].

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References and Notes

(1) ICMIB is associated with the CNR National Institute for the Chemistry of Biological System (CBS).
(2) Faulkner, D. J.; Ghiselin, M. T. *Mar. Ecol. Prog. Ser.* **1983**, *13*, 295−301.
(3) Karuso, P. *Biorganic Marine Chemistry*; Scheuer, P., Ed.; Springer: New York, 1989; p 31.
(4) Cimino, G.; Sodano, G. *Chem. Scripta* **1989**, *29*, 389−394.
(5) Fontana, A.; Avila, C.; Martinez, E.; Ortea, J.; Trivellone, E.; Cimino, G. *J. Chem. Ecol.* **1993**, *19*, 339−356.
(6) Fontana, A.; Jimenez, F.; Marin, A.; Mollo, E.; Cimino, G. *Experientia* **1994**, *50*, 510−516.
(7) Garcia-Gómez, J. C.; Cimino, G.; Medina, A. *Marine Biology* **1990**, *106*, 245−250.
(8) Quilico, A.; Piozzi, F.; Pavan, M. *Tetrahedron* **1957**, 177−185.
(9) Cimino, G.; De Stefano, S.; Guerriero, A.; Minale, L. *Tetrahedron Lett.* **1975**, 1425−1428.
(10) Thompson, J. E.; Walker, R. P.; Wratten, S. J.; Faulkner, D. J. *Tetrahedron* **1982**, *38*, 1865−1873.
(11) Avila, C. *Ocean. Mar. Biol. Annu. Rev.* **1995**, *33*, 487−559.
(12) Fontana, A.; Mollo, E.; Ricciardi, D.; Fakr, I.; Cimino, G. *J. Nat. Prod.* **1997**, *60*, 444−448, and reference therein.
(13) Kazlauskas, R.; Murphy, P. T.; Wells, R. J.; Noack, K.; Oberhänsli, W. E.; Schönholzer, P. *Aust. J. Chem.* **1979**, *32*, 867−880.
(14) Fontana, A.; Gavagnin, M.; Mollo, E.; Ortea, J.; Cimino, G. *Comp. Biochem. Physiol.* **1995**, *111B*, 283−290.
(15) Muniaín, C.; Valdés, A.; Ortea, J. *J. Moll. Stud.* **1996**, *62*, 265−273.

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