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New Acetoxy-*ent*-Pallescensin-A Sesquiterpenoids from the Skin of the Porostome Nudibranch *Doriopsilla areolata*

Aldo Spinella,[1] Luis A. Alvarez,[2] Conxita Avila,[3] and Guido Cimino

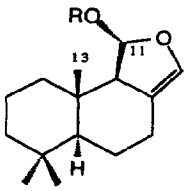
Istituto per la Chimica di Molecole di Interesse Biologico, C. N. R.
Via Toiano, 6 - 80072 Arco Felice (NA) - Italy.

Abstract: The paper reports the first chemical study of an European *Doriopsilla* mollusc. Three new furanosesquiterpenoids, *ent*- pallescensin-A (2) together with two acetoxy derivatives (5,6), have been characterized. The metabolites are distributed either in the skin of the mollusc or in its hermaphrodite gland where the most abundant component is a mixture of drimane esters (1).

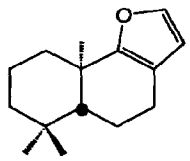
Porostome nudibranchs are marine molluscs without the radula. Because of this these opisthobranchs can only feed by sucking soft tissues. The superfamily Porodoridoidea includes two families, Phyllidiidae and Dendrodorididae.[4] The latter is further split in two genera, *Dendrodoris* and *Doriopsilla*. Extensive studies were performed with *Dendrodoris* molluscs,[5] whereas only one paper reports chemical studies of *Doriopsilla* nudibranchs.[6] However, the metabolite patterns are closely related, both molluscs contain in their internal glands a series of fatty acid esters (1) of the same drimane sesquiterpenoid, whereas related drimane metabolites have been found in the external tissues where they play a defensive role. Now we will report the first chemical study of a nudibranch belonging to *Doriopsilla* genus, *D. areolata*, from the European coasts.

Doriopsilla areolata Bergh, 1880 was collected in different areas of the Mediterranean Spanish coast (Algeciras; Blanes) at a depth of ten meters by SCUBA divers. All collections were characterized by the same metabolite pattern. The mantle acetone extracts (157 mg from 44 molluscs) displayed two Ehrlich positive spots (*Rf* 0.5 and 0.2; TLC SiO_2; *n*-hexane/ethyl acetate, 90:10) whereas the viscera extracts (308 mg from 44 molluscs) revealed, analogously with the previous studies of Pacific species, some drimane fatty acid esters (1 ; 47.7 mg) together with two apolar Ehrlich positive metabolites 2 and 3. After isolation, 2 (1.0 mg) and 3 (1.2 mg) exhibited the already described ^{1}H-NMR spectra of pallescensin-A [7] and euryfuran (3).[8] The latter ($[\alpha]_D^{25}$ + 20.0°; c 0.12, $CHCl_3$) might be a work-up artifact from the drimane esters. Surprisingly, the $[\alpha]_D^{25}$ value of 2 (- 73.8°; c 0.05, $CHCl_3$) was opposite to that of (+)- pallescensin-A (4), suggesting an enantiomeric relationship with the already known natural product, that was confirmed by recording CD spectra.[9]

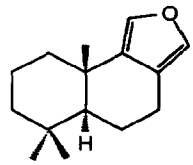
The ^{1}H-NMR spectra, together with the elemental composition, suggested a structure related to pallescensin-A for both the Ehrlich positive compounds (5, 6) from the mantle. The molecular formula for the less polar compound 5 (16.1 mg; IR 1733 cm^{-1}; $[\alpha]_D^{25}$ - 53.5°; c 0.74, $CHCl_3$) was established as $C_{17}H_{24}O_3$ by HRMS of the molecular peak at *m/z* 276.1721 (calc. 276.1725). In the ^{1}H-NMR spectrum the protons of an α,β disubstituted furan system were recorded at δ 7.18 (H-12) and 6.11 (H-11), but only two



1 R = acyl residues from several fatty acids



2



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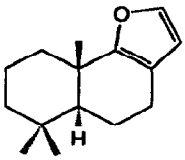

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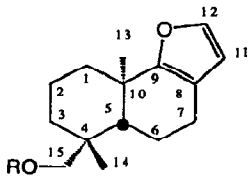
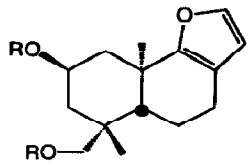
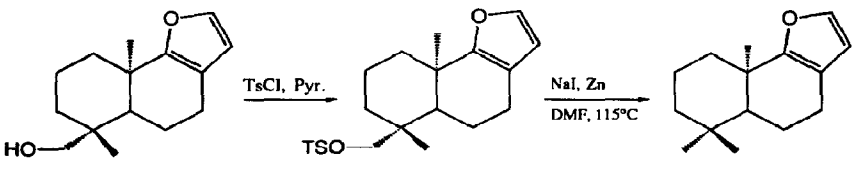


5 R = CH_3CO
7 R = H



6 R = CH_3CO
8 R = H

angular methyls were observed at δ 1.20 (H_3-13) and 1.02 (H_3-14), whereas an isolated AB system with resonances centered at δ 4.24 and 4.01 (H_2-15, J= 11.1 Hz) and a methyl singlet at δ 2.06 well fitted for the presence of an angular CH_2OAc system. 2D-NMR experiments (^{1}H-^{1}H COSY, ^{1}H-^{13}C COSY and HMBC) allowed us to assign all ^{1}H and ^{13}C resonances (Table 1). The methylenacetoxy group was axially oriented according to the ^{13}C-NMR values of C-15 (δ 67.2) and C-5 (δ 52.8).[10] In addition, the ^{1}H-NMR singlet at δ 1.20, correlated with the carbon at δ 21.6, was assigned to the protons (H_3-13) of the methyl near the furan ring, whereas the ^{13}C-NMR downfield chemical shift of the second methyl (C-14, δ 27.6) suggested an equatorial orientation. These assignments were confirmed by the correlations observed in the HMBC (J = 10 Hz) 2D-NMR spectrum. Thus, the methyl protons at δ 1.02 (H_3-14) showed long-range couplings with carbons at δ 67.2 (C-15), δ 52.8 (C-5) and δ 36.6 (C-3) while the methyl group resonating at δ 1.20 (H_3-13) was long-range coupled with the signals at δ 52.8 (C-5) and δ 35.6 (C-1). The structural relationship with *ent*-pallescensin-A (**2**) was suggested by the negative CD maxima (EtOH) at 230 nm (Θ -2457) and 215 nm (Θ -2842) recorded for the deacetyl derivative **7** [11] obtained by methanolysis (MeOH an., Na_2CO_3, 3h) of **5**, and confirmed by a simple chemical degradation. The compound **7** (3 mg), dissolved in $CHCl_3$ an., was tosylated by addition of *p*-toluenesulfonyl chloride and pyridine (r.t., 6 days). The tosylate **9** (2 mg), dissolved in DMF, was reduced [12] by treatment with NaI and activated Zn (115 °C, 6 h), affording 0.5 mg of a product identical in all aspects (TLC, CD, ^{1}H-NMR) to *ent*-pallescensin-A (**2**).



7 **9** **2**

Table 1. ^{1}H- and ^{13}C-NMR° Data for Compounds **5** and **6**.

	5				**6**			
Position	δ ^{1}H	m, *J*(Hz)	δ ^{13}C	m	δ ^{1}H	m, *J*(Hz)	δ ^{13}C	m
1	2.15 1.40	bd, *13.3* ddd, *13.3; 13.2; 3.8*	35.6	t	2.16 1.18	ddd, *12.8; 4.5; 2.0* dd, *12.8; 12.6*	41.4[b]	t
2	1.68 1.58	m m	18.3	t	5.13	dddd. *12.6; 11.9; 4.5; 2.5*	67.6	d
3	1.77 1.10	m ddd, *13.8; 14.0; 4.1*	36.6	t	2.52 1.43	ddd, *11.9; 2.5; 2.0* dd, *11.9; 11.9*	40.7[b]	t
4	-		36.4[a]	s	-		37.4[c]	s
5	1.53	dd, *12.5; 1.2*	52.8	d	1.57	dd, *12.0; 1.2*	52.2	d
6	1.96 1.60	dddd,*13.1; 6.2; 1.2;1.0*	20.0	t	1.97 1.61	bddd, *12.8; 6.3; 1.2* m	19.6	t
7	2.47 2.34	ddd, *15.8; 5.7; 1.0* ddd, *15.8; 11.3; 6.2*	23.1	t	2.49 2.36	bdd, *16.0; 5.0* ddd, *16.0; 10.8; 6.3*	22.9	t
8	-		113.7	s	-		113.8	s
9	-		159.1	s	-		157.8	s
10	-		36.8[a]	s	-		38.4[c]	s
11	6.11	d, *1.8*	110.2	d	6.12	d, *1.8*	110.0	d
12	7.18	d, *1.8*	140.2	d	7.18	d, *1.8*	140.6	d
13	1.20	s	21.6	q	1.27	s	22.6	q
14	1.02	s	27.6	q	1.09	s	27.7	q
15	4.24 4.01	d, *11.1* d, *11.1*	67.2	t	4.18 4.06	d, *11.2* d, *11.2*	67.0	t
$\underline{CH_3}CO$	2.06	s	21.0	q	2.08	s	21.3	q
$CH_3\underline{CO}$	-		171.2	s	-		171.1	s
$\underline{CH_3}CO$					2.04	s	20.9	q
$CH_3\underline{CO}$					-		170.4	s

° Bruker 500 AMX; $CDCl_3$; chemical shifts referred to $CHCl_3$ at 7.26 ppm and to $CDCl_3$ at 77.0 ppm.
[a,b,c] Values with identical superscript in the same column could be interchanged.

Compund **6** (2,15-diacetoxy-*ent*-pallescensin-A; 3.2 mg; $[\alpha]_D^{25}$ - 26.8°, c 0.25, $CHCl_3$; $C_{19}H_{26}O_5$ HRMS of M^+ at *m/z* 334.1783, calc. 334.1780) showed, analogously with **5**, a band at 1732 cm^{-1} in the IR spectrum. The ^{1}H-NMR spectrum of this more polar metabolite exhibited analogies with the above described data of **5**. However, a second singlet at δ 2.08 was assigned to the methyl of a secondary acetoxy group linked to C-2. The ^{1}H-NMR multiplicity of H-2 (δ 5.13, dddd; *J* (Hz) =12.6; 11.9; 4.5; 2.5) agreed with an axial orientation, that was confirmed by some diagnostic n.O.e. experiments. In fact, irradiation at δ 5.13 caused nuclear Overhauser enhancements of the signals at δ 2.52 (H-3eq) and δ 1.27 (H_3-13) while in another n.O.e. experiment positive effects on H-2, H_3-14 and H_3-13 were induced by irradiating at δ 4.06 (H-15). This confirmed the equatorial orientation of the methylenacetoxy group at C-2. Of course all ^{1}H and ^{13}C NMR resonances (Table 1) were assigned by careful analysis, which included 2D-NMR experiments (^{1}H-^{1}H COSY, ^{1}H-^{13}C COSY, HMBC).The CD curve of the deacetylated compound **8**,[13] obtained by methanolysis of **6**, exhibited the same negative profile of **7** so assigning the same absolute stereochemistry.

The mixture of drimane esters (**1**)[14] was structurally characterized by comparison of the ^{1}H-NMR data with those reported for the related mixtures from Pacific porostome nudibranchs [6] and from Mediterranean *Dendrodoris* molluscs.[15] The absolute stereochemistry of **1** was inferred through elimination

of the acyl residues by heating a solution of **1** (30 mg) in petroleum ether with silica gel. This transformation yielded 12 mg of **3** ($[\alpha]_D^{25}$ + 21.7 °, c 1.0, $CHCl_3$). The β orientation of H-11 (δ 6.32) was supported by a positive n.O.e. with H_3-13 (δ 0.81). The co-occurrence in the same organism of sesquiterpenoids with opposite A/B ring fusion is quite unusual. However, the absolute stereochemistry of compounds **2** and **3** was strongly supported by the comparison of their $[\alpha]_D$ values with those recorded in a series of authoritative syntheses.[7b,8b]

Preliminary anatomical dissections of *D. areolata* showed that **5** and **6** are concentrated along the border of the mantle whereas **2** is located in the mantle and in the hermaphrodite gland. Analogously with *Dendrodoris limbata* and *Dendrodoris grandiflora*,[16] the mixture **1** is present only in the hermaphrodite gland of *D. areolata* whereas it is completely absent in other internal glands. The absence of drimane sesquiterpenoids, as olepupuane and polygodial until now found in all studied porostome nudibranchs,[5] in the external tissues of the molluscs is very intriguing. Probably, the biological function against potential predators is played in *D. areolata* by the acetoxy-derivatives (**5-6**) of *ent*-pallescensin-A. It was important to exactly localize (-)-*ent*-pallescensin-A (**2**) in order to obtain information about its origin. Unfortunately, biosynthetic studies are difficulty to plan having found, until now, only populations with very few individuals. However, even though (+) - pallescensin-A (**4**) is a known sponge metabolite,[7] the absence of **2** in the digestive glands of *D. areolata* should support its "de novo" biosynthesis.

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11. **7** ^{1}H-NMR ($CDCl_3$) δ: 7.18 (1H, d 1.7 Hz); 6.10 (1H, d 1.7 Hz); 3.78 (1H, dd 10.9, 5.7 Hz); 3.58 (1H, dd 10.9, 4.6 Hz); 2.47 (1H, ddd 15.9, 4.6, 1.1 Hz); 2.34 (1H, ddd 15.9, 11.2, 6.2 Hz); 2.14 (1H, bd 13 Hz); 1.96 (1H, dd 12.9, 6.2 Hz); 1.41 (1H, ddd 13.0, 13.2, 3.8 Hz); 1.19 (3H, s); 1.04 (3H, s).
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13. **8** ^{1}H-NMR ($CDCl_3$) δ: 7.19 (1H, d 1.6 Hz); 6.11 (1H, d 1.6 Hz); 4.04 (1H, m); 3.73 (1H, bd 10.8 Hz); 3.58 (1H, bd 10.8 Hz); 2.52 (1H, m); 2.48 (1H, bdd 16.0, 5.5 Hz); 2.36 (1H, ddd 16.0, 10.8, 6.0 Hz); 2.22 (1H, dd 13.0, 2.0 Hz); 1.35 (1H, dd 11.9,11.9 Hz); 1.22 (3H, s); 1.11 (3H, s); 1.02 (1H, m).
14. Methanolysis of esters **1**, obtained from the hermaphroditc glands of *D. areolata*, gave a mixture of fatty acids methyl esters. This mixture was analyzed by glc (25 m glass capillary OV-1 column), the most abundant identified components being $C_{16:0}$ (15.3 %), $C_{18:0}$ (11.4 %), $C_{20:0}$ (2.0 %), $C_{16:1}$ (4.0 %), $C_{18:1}$ (9.1 %) , $C_{20:1}$ (1.4 %) .
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