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Diasteroisomeric ichthyotoxic acylglycerols from the dorsum of two geographically distinct populations of *Archidoris* nudibranchs

E. Zubía[1], M. Gavagnin, A. Crispino, E. Martínez[a], J. Ortea[a] and G. Cimino

Istituto per la Chimica di Molecole di Interesse Biologico del C.N.R., Via Toiano 6, I-80072 Arco Felice, Napoli (Italy), and [a]Departamento de Biología de Organismos y Sistemas, Universidad de Oviedo, C/J. Arias de Velasco, Oviedo (Spain)

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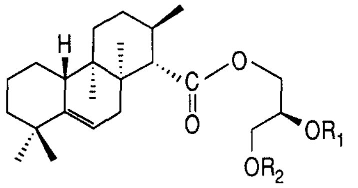
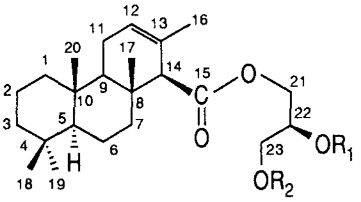
Abstract. Two marine opisthobranchs, *Archidoris tuberculata* from Asturias (N. Spain) and *Archidoris carvi* from Patagonia (S. Argentina), contain in their dorsum diasteroisomeric ichthyotoxic acylglycerols esterified in position 1-*sn* by antipodal diterpenoid acids.

Key words. Acylglycerols; ichthyotoxins; nudibranchs; marine molluscs.

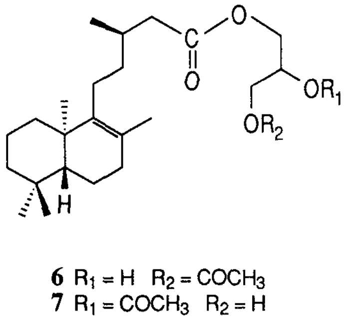
Chemical studies of the defensive strategies of the apparently unprotected marine nudibranchs have discovered many ecologically relevant molecules[2,3], which not only play a part in defensive strategies but often possess other basic biological functions[4]. Among recent examples are studies of the multifunctional drimane sesquiterpenoids from Dendrodorididae species[5], of prostaglandin lactones from *Tethys fimbria*[6] and, finally, of some diacylglycerols, verrucosins (**1**, **2**), from *Doris verrucosa*[7]. The interesting biological properties of verru-



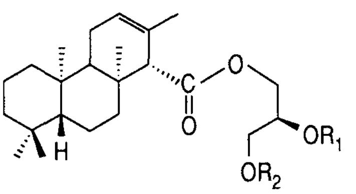
1 $R_1 = H$, $R_2 = COCH_3$
2 $R_1 = COCH_3$, $R_2 = H$



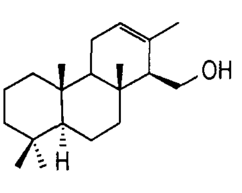
3 $R_1 = H$, $R_2 = COCH_3$
4 $R_1 = COCH_3$, $R_2 = H$
5 $R_1 = R_2 = H$



6 $R_1 = H$ $R_2 = COCH_3$
7 $R_1 = COCH_3$ $R_2 = H$



8 $R_1 = H$ $R_2 = COCH_3$
9 $R_1 = COCH_3$ $R_2 = H$
10 $R_1 = R_2 = H$



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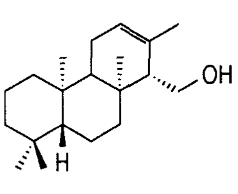

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cosins (**1**, **2**), which are highly ichthyotoxic to *Gambusia affinis*[8,9] and are activators of protein kinase C[10], could be due to their unusual structure[7,11], featuring a rearranged isocopalane[12] diterpenoid ester in position C-1 of *sn*-glycerol. Structurally related acyl glycerols were described previously from British Columbian *Archidoris montereyensis*[13,14] and *Archidoris odhneri* (**3–5**)[14], and more recently from Antarctic *Austrodoris kerguelensis* (**6**, **7**)[15]. Even though biosynthetic experiments were successful only with *A. montereyensis* and *A. odhneri*, it seems an attractive hypothesis that there is a small group of Eudoridoidea nudibranchs, belonging to closely related families, which is able to produce potentially defensive allomones by de novo biosynthesis of acylglycerols.

In this paper we report studies on two further Eudoridoidea nudibranchs, *Archidoris tuberculata* (Müller, 1778) and *Archidoris carvi* (Marcus, 1955). The former possesses the same metabolites (**3–5**) as *A. montereyensis*, whereas *A. carvi* contains two new glycerides (**8**, **9**) characterized by a diterpenoid residue enantiomeric with that of **3–5**.

Specimens of *A. tuberculata* (16 individuals) were collected at Rio del Eo (Asturias, North Spain) in the intertidal zone from December, 1990, to April, 1991. Samples were immediately frozen and stored at low temperature until February, 1992. Mantles and viscera were separately extracted with acetone. The TLC (SiO_2, *n*-hexane/diethyl ether, 1:1) chromatographic pattern of the diethyl ether-soluble fraction of the acetone extract of the mantles was dominated by two compounds (*Rf* 0.35 and 0.30) which were completely absent in the extract of the viscera. Subsequent separation on a preparative column (SiO_2, petroleum ether/diethyl ether gradient) and by HPLC (μ-Porasil, *n*-hexane/ethyl acetate, 85:15) led to two compounds (5.5 mg and 2.5 mg) identical to **3** and **4**, already described[14]. Superior NMR facilities which became available later allowed more complete assignments (table) which agreed well for the diterpenoid part with the NMR values previously assigned[16]. Chromatography also led to the isolation of a more polar compound identical with **5**, and of a less polar acylglycerol (*Rf* 0.6, TLC), probably esterified by a bicyclic diterpenoid acid residue, whose structure remains to be determined.

Specimens of *A. carvi*[17] (7 individuals) were collected at Punta Pardelas (Peninsula of Valdes, South Argentina) by snorkeling, at a depth of 1–10 m during November, 1991. The molluscs were stored, dissected and analyzed analogously to *A. tuberculata*. The ether-soluble fraction (80 mg) from the acetone extract of the mantles displayed a TLC chromatographic pattern almost identical with that of the corresponding extract from *A. tuberculata*. After two chromatographic steps [(1)SiO_2 column, gradient of *n*-hexane/diethyl ether mixtures; (2)μ-Porasil, *n*-hexane/diethyl ether, 85:15] two diacylglycerols **8** (2.2 mg) and **9** (0.8 mg) were obtained.

Table. ^{1}H- and ^{13}C-NMR data of **3**, **4**, **8** and **9**[a]

	δ^{1}H[b]				δ^{13}C(m)[b]	
C	**3**	**4**	**8**	**9**	**3**	**8**
1	0.80 1.61	0.80 1.61	0.80 1.62	0.80 1.62	39.84(t)	39.85(t)
2	1.38 1.58	1.38 1.58	1.38 1.57	1.38 1.57	18.60*(t)	18.61*(t)
3	1.14 1.38	1.14 1.38	1.14 1.38	1.13 1.38	41.82(t)	41.83(t)
4	-	-	-	-	33.14(s)	33.0(s)
5	0.84	0.84	0.85	0.85	56.41(d)	56.42(d)
6	1.38 1.58	1.38 1.58	1.38 1.57	1.38 1.57	18.44*(t)	18.45*(t)
7	1.38 1.70	1.38 1.68	1.38 1.70	1.38 1.68	41.82(t)	41.83(t)
8	-	-	-	-	37.39(s)	37.5(s)
9	1.16	1.15	1.16	1.15	54.24(d)	54.24(d)
10	-	-	-	-	36.58(s)	36.5(s)
11	1.95	1.95	1.95	1.95	22.64(t)	22.65(t)
12	5.53	5.53	5.53	5.52	124.32(d)	124.36(d)
13	-	-	-	-	128.45(s)	128.46(s)
14	2.96	2.95	2.96	2.94	62.51(d)	62.51(d)
15	-	-	-	-	173.02(s)	§
16	1.60	1.60	1.60	1.60	21.16(q)	21.19(q)
17	0.94*	0.93*	0.95*	0.93*	15.57(q)	15.56(q)
18	0.81	0.83	0.81	0.81	21.64(q)	21.64(q)
19	0.86	0.86	0.86	0.86	33.40(q)	33.41(q)
20	0.90*	0.90*	0.90*	0.90*	15.72(q)	15.72(q)
21	4.12 4.22[c]	4.27 4.34	4.12–4.24[c]	4.23–4.35	64.83(t)	64.92(t)
22	4.10	5.07	4.12	5.10	68.37(d)	68.40(d)
23	4.12 4.22[c]	3.76	4.12–4.24[c]	3.76	65.30(t)	65.29(t)
OCOMe	2.11	2.11	2.11	2.10	20.77(q)	20.78(q)

[a] Bruker AMX-500 spectrometer; $CDCl_3$; chemical shifts referred to $C\underline{H}Cl_3$ at 7.26 ppm and to $\underline{C}DCl_3$ at 77.00 ppm.
[b] The assignments were aided by ^{1}H-^{1}H COSY and ^{1}H-^{13}C HETCOR. [c] Unresolved 4 proton multiplets. §Not detected.
*Starred values in the same column can be interchanged.

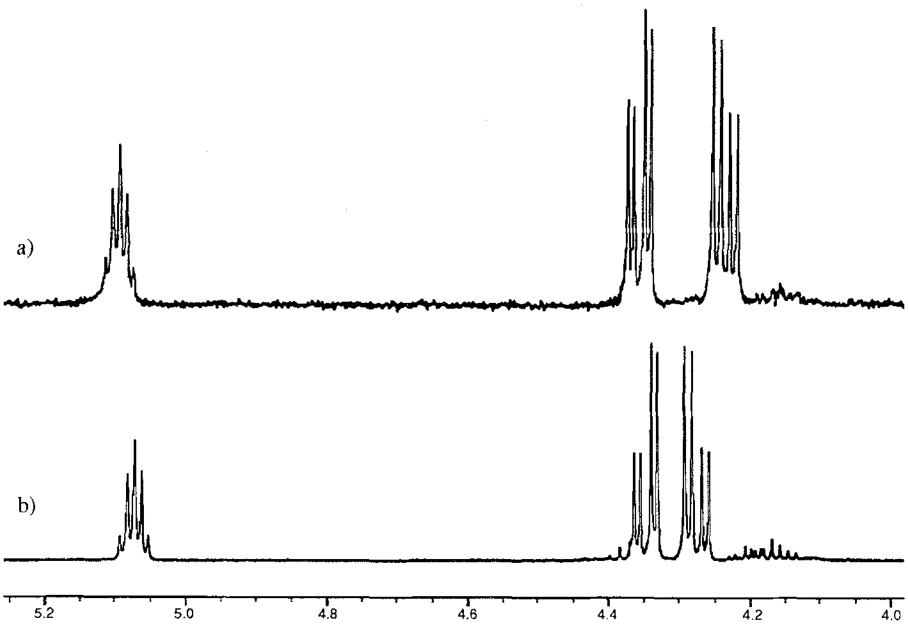

Figure 1. Partial ^{1}H-NMR of **9**(a) and **4**(b).

The spectral data of **8** and **9**[18] were almost identical to those of **3** and **4**. However, detailed careful comparison of the ^{1}H-NMR spectra of **4** and **9** revealed diagnostic differences (fig. 1) in the chemical shifts of the H-21 and H-22.

Similarly, **3** and **8** exhibited characteristic ^{1}H-NMR profiles which were different, but too complex to analyze as all glycerol protons resonate between δ 4.10 and 4.24. Fortunately, slight shifts were observed in their ^{13}C-NMR spectra for C-21 (δ 64.83 for **3** and δ 64.92 for **8**) (table). These data supported diasteroisomeric relationships between the two pairs **3**–**8** and **4**–**9**, which could be due to opposite absolute stereochemistry, either of the diterpenoid acid or of the glycerol moiety. While the optical rotations of **3** and **4** are negative, as already reported[14], the corresponding values for **8**, $[\alpha]_D + 21.9°$ (c = 0.22, $CHCl_3$), and **9**, $[\alpha]_D + 66.9°$ (c = 0.07, $CHCl_3$), were both positive, thereby suggesting opposite stereochemistry of the diterpenoid residue. The CD spectra (fig. 2) displayed at the same wavelength ($\sim$214 nm) negative curves for **3**, **4** and **5**, and positive for **8**, **9** and their deacetyl derivative **10**. Oxidation of **3** and **8** with Jones' reagent yielded ketones displaying identical ^{1}H-NMR [0.82, 0.86, 0.91, 0.95, 2.18 (s, 3H each); 3.04 (bs, H-14); 4.75 (m, H-21 and H-23), 5.55 (m, H-12)] spectra but coherent opposite CD profiles. Finally, reduction of **3** and **8** with $LiAlH_4$ yielded alcohol **11**[13,19], $[\alpha]_D - 12°$ (c = 0.25, $CHCl_3$); lit. $[\alpha]_D - 9°$, and its enantiomer **12**, $[\alpha]_D + 10°$ (c = 0.04, $CHCl_3$), thus confirming the suggested isocopalane skeleton of the diterpenoid acid residue of **8**[20].

It is worth noting that probably only **4** and **9** are natural products, whereas **3** and **8** might arise from an

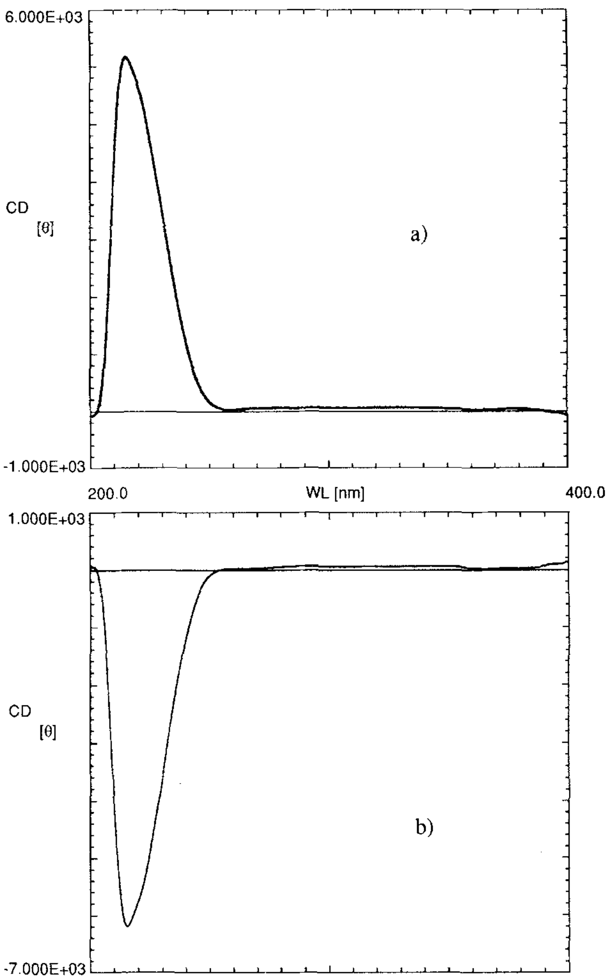

Figure 2. CD spectra of **8**(a), $[\theta]_{214} = 5412$, and **3**(b), $[\theta]_{214} = -6195$.

easy *trans*-acetylation. However, bearing in mind the stereospecific numbering for the derivatives of glycerols, both **4** and **9** are 1,2-diacyl-*sn*-glycerols. Enantiomeric terpenoids are rather common among sesquiterpenoids[21], relatively rare among diterpenoids[22], and extremely rare among the superior terpenoids where there are also some inexact reports, e.g. *ent*- 12-*epi*-deoxoscalarin[23].

In conclusion, it seems that *Archidoris*, *Doris* and *Austrodoris* nudibranchs have elaborated a defensive strategy which utilizes acylglycerols as defensive allomones. Preliminary bioassays have revealed strong ichthyotoxicity of **3** and **4** at 10 and 1 ppm against *Gambusia affinis*.

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