New Minor Diterpenoid Diacylglycerols from the Skin of the Nudibranch *Anisodoris fontaini*

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The Patagonian dorid nudibranch *Anisodoris fontaini* contains in its mantle a series of isocopalane diterpenoid diacylglycerols. Five new minor metabolites, anisodorins 1−5 (**1**−**5**), along with the already reported **6** and **7**, have been isolated and chemically characterized. The structure and the relative stereochemistries have been determined by spectroscopic means, while the absolute stereochemistries for **2**−**5** are suggested to be the same as for the biogenetically related major compounds **6** and **7**. Synthesis of the enantiomer (**8**) of anisodorin 1 confirmed the proposed structure and absolute stereochemistry.

Opisthobranchs are marine mollusks that have elaborated some defensive strategies[1] because they are poorly protected by their shells. These mollusks often possess unusual molecules, which can play a defensive role against potential predators[2,3] and which also possess other biological functions.

Dorid nudibranchs belonging to the genera *Archidoris*, *Doris*, and *Austrodoris* have been found to contain in their mantles terpenoid glyceryl esters, displaying very interesting biological activities. De novo biosynthesis of diacylglycerols in *Archidoris* species has been also demonstrated.[4]

In the course of our study of the chemical ecology of opisthobranch mollusks, which has been focused recently on dorid nudibranchs, we have characterized a series of ichthyotoxic 1,3-*sn* and 1,2-*sn* diterpenoid diacyglycerols,[5−9] which have been shown to be potent activators of protein kinase C and to be very active in a regenerative test with the freshwater hydrozoan *Hydra vulgaris*.[10] Among the nudibranchs studied, the Patagonian dorid *Anisodoris fontaini* d'Orbigny, 1837 (order Nudibranchia, family Dorididae) erroneously classified in the previous report[6] as *Archidoris carvi*) was found to contain two main glyceryl esters **6** and **7**, also isolated from the Mediterranean *Doris verrucosa*,[9] which are diastereoisomers of two diacylglycerols found in another dorid nudibranch, *Archidoris tuberculata*, collected along the northern Spanish coast.[6] Surprisingly, the isomers from each source display opposite absolute stereochemistry in their terpenoid moiety.

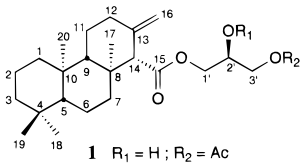
Even though biosynthetic experiments[4] have proved that related mollusks from the Pacific Ocean are able to biosynthesize acylglycerols *de novo*, no direct experimental evidence has been collected until now for dorids from other geographical regions. Because of this, we have investigated *A. fontaini* further in order to better understand, through the structural features of their minor metabolites, the origin and biological role of this interesting group of bioactive molecules.

In this paper, we report the chemical characterization of five further novel diterpenoid metabolites, anisodorins 1−5 (**1**−**5**), four of which are glyceryl ester derivatives, that have been isolated from the skin of *A. fontaini*.

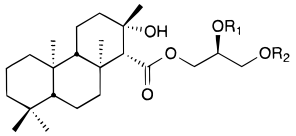
Results and Discussion

Specimens of *A. fontaini* (30 individuals) were collected along the Patagonian coast (southern Argentina), immediately frozen, and stored at −20 °C until they were transferred to ICMIB. After some months, the animals were treated with acetone by using ultrasonic vibration, to extract only the metabolites present in the external part of the nudibranch. Then the mollusks were again extracted with acetone. The Et_2O-soluble fractions of both acetone extracts were compared by TLC, revealing the presence of a series of compounds (R_f 0.45−0.30, petroleum ether−Et_2O, 1:1), exclusively in the extract of the external part of the mollusk.

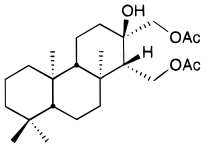
The ultrasonic Et_2O extract (213 mg) was therefore chromatographed on a Si gel column using an elution gradient from petroleum ether to Et_2O. The fractions containing anisodorins were purified by normal-phase HPLC, giving anisodorin 1 (**1**, 1.4 mg), anisodorin 2 (**2**, 1.0 mg), anisodorin 3 (**3**, 1.3 mg), anisodorin 4 (**4**, 2.0 mg), and anisodorin 5 (**5**, 1.5 mg), along with the already reported major diacylglycerols[6] **6** (22.8 mg) and **7** (9.1 mg).



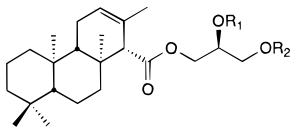
1 R_1 = H ; R_2 = Ac
2 R_1 = Ac ; R_2 = H



3 R_1 = H ; R_2 = Ac
4 R_1 = Ac ; R_2 = H



5 *



6 R_1 = H ; R_2 = Ac
7 R_1 = Ac ; R_2 = H

* relative stereochemistry

The ^1H and ^{13}C NMR spectra of compounds **1**−**5** showed strong similarities with those of the major compounds **6** and **7**, indicating that the new anisodorins were closely related structurally to these known metabolites, particularly in having the same isocopalane skeleton. In addition,

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Table 1. ^{1}H NMR Data[a,b] of Anisodorins 1−5 (**1**−**5**)

	δ ^{1}H m, *J* (Hz)				
position	**1**	**2**	**3**	**4**	**5**
1	0.80 m	0.81 m	0.80 m	0.80 m	0.83 m
	1.65 m	1.65 m	1.72 m	1.72 m	1.68 m
2	1.40 m	1.40 m	1.40 m	1.40 m	1.40 m
	1.60 m	1.60 m	1.60 m	1.60 m	1.60 m
3	1.14 ddd, 4.5, 13.4, 13.8	1.14 ddd, 4.0, 13.3, 14.4	1.12 ddd, 8.7, 13.2, 13.4	1.12 m	1.15 m
	1.38 m	1.38 m	1.36 m	1.36 m	1.38 m
5	0.85 m	0.87 m	0.84 m	0.84 m	0.82 m
6	1.40 m	1.40 m	1.40 m	1.40 m	1.58 m
	1.60 m	1.60 m	1.50 m	1.50 m	
7	1.31 ddd, 2.4, 10.0, 12.8	1.30 m	1.30 m	1.30 m	1.18 m
	1.60 m	1.60 m	1.45 m	1.45 m	1.72 m
9	1.05 dd, 2.7, 12.6	1.04 m	0.83 m	0.83 m	0.96 ddd, 1.8, 12.1
11	1.42 m	1.40 m	1.50 m	1.50 m	1.24 m
	1.68 m	1.70 m	1.65 m	1.65 m	1.65 m
12	2.03 ddd, 5.1, 12.5, 13.2	2.04 m	1.28 m	1.28 m	1.36 m
	2.40 m	2.40 ddd, 2.1, 2.2, 13.4	1.87 ddd, 3.1, 3.2, 13.7	1.86 ddd, 3.1, 3.1, 13.6	2.05 m
14	2.84 s	2.82 s	2.15 s	2.13 s	1.70 dd, 3.6, 6.0
15					4.20 dd, 6.0, 11.9
					4.52 dd, 3.6, 11.9
16	4.66 d, 1.1	4.66 d, 1.2	1.11 s	1.10 s	4.02 d, 11.7
	4.82 br s	4.82 d, 1.0			4.25 br d, 11.7
17	1.06 s	1.05 s	1.18 s	1.16 s	0.89 s
18	0.80 s	0.81 s	0.80 s	0.80 s	0.81 s
19	0.86 s	0.86 s	0.85 s	0.84 s	0.86 s
20	0.84 s	0.84 s	0.87 s	0.86 s	0.81 s
OH-13			3.59 d, 2.4	3.62 d, 2.4	3.03 s
OAc-15					2.06 s
OAc-16					2.10 s
1′	4.10−4.20 m	4.28 dAB quartet, 4.6, 12.2	4.18 dd, 4.3, 11.5	4.26 dd, 6.1, 12.0	
			4.26 dd, 4.0, 11.5	4.40 dd, 4.0, 12.0	
2′	4.08 m	5.07 app. quintet, 5.0	4.11 m	5.12 m	
3′	4.10−4.20 m	3.74 dd, 5.5, 6.1	4.16 m	3.78 m	
−OAc	2.10 s	2.10 s	2.11 s	2.10 s	
−OH	2.41 d, 5.0		2.38 d, 4.7		

[a] Bruker AMX 500 MHz, $CDCl_3$, chemical shifts (ppm) referred to C*H*Cl_3 (δ 7.26). [b] Assignments made by ^{1}H−^{1}H COSY, HMQC, and ^{1}H−^{1}H homodecoupling experiments.

anisodorins 1−4 (**1**−**4**) showed, by analogy with **6** and **7**, a diacylglycerol structure, containing a monoacetylated glyceryl fragment linked to a diterpenoid acid.

Anisodorin 1 (**1**), isolated as oil, exhibited a molecular formula $C_{25}H_{40}O_5$, deduced from both its EIMS and ^{13}C NMR data. The ^{1}H NMR spectrum displayed four methyl singlets at δ 0.80, 0.84, 0.86, and 1.06 and two methine signals at δ 4.66 (d, J = 1.1 Hz) and 4.82 (br s), supporting the presence of the same skeleton as **6**, containing in ring C an exomethylene group in the place of a vinyl methyl at C-13. A series of signals in the ^{1}H NMR spectrum at δ 4.10−4.20 (m, 4 H) and 4.08 (m, 1 H) and in the ^{13}C NMR spectrum at δ 64.6 (t), 65.3 (t), and 68.3 (d) revealed a 1,3-diacylglycerol structure. ^{1}H and ^{13}C NMR values, completely assigned by 1D and 2D NMR experiments (Tables 1 and 2), were consistent with the suggested structure **1**. The absolute stereochemistry of the diterpenoid part of **1** should be the same as **6**, the major skin metabolite of *A. fontaini*, on the basis of biogenetic considerations. The CD profile of **1**, identical to that of **6**, confirmed this hypothesis.[6,9] The absolute stereochemistry at C-2′ of the glyceryl moiety was suggested to be *S*, the same as that of the known diacylglycerols **6** and **7**.

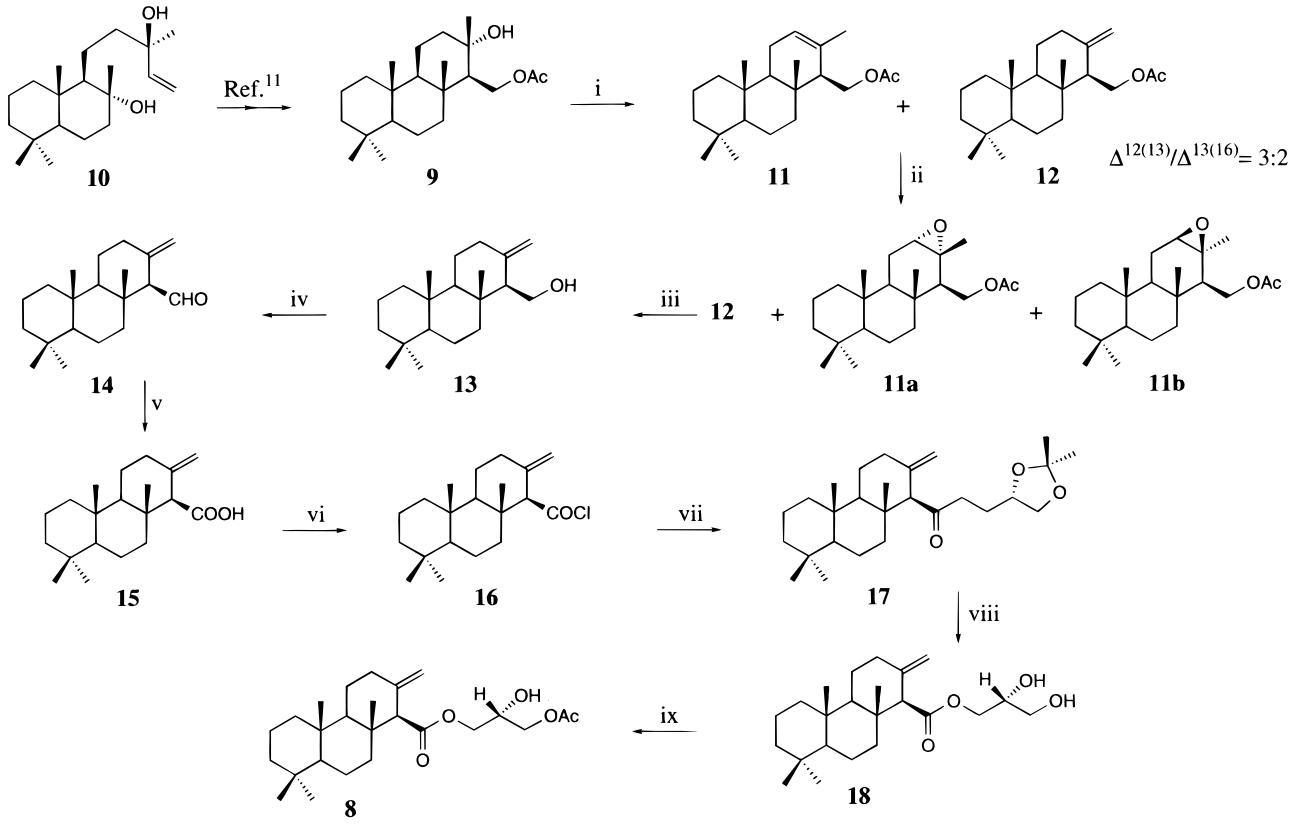
To further confirm the proposed structure, a synthesis of the enantiomer of **1** (**8**) was carried out (Scheme 1), starting from the *ent*-isocopalane hydroxyacetate derivative **9**, obtained from sclareol (**10**), according to a literature procedure.[11]

The treatment of **9** (in dry pyridine solution) with phosphorus oxychloride, under Ar, gave in high yield (88%) a mixture of the two possible dehydration products: the $\Delta^{12(13)}$ (**11**) and the $\Delta^{13(16)}$ (**12**) isomers (3:2). To separate the two isomers, which showed the same chromatographic behavior, the mixture was treated with monoperphthalic acid, with stirring. The $\Delta^{12(13)}$ isomer (**11**) reacted quantitatively, giving a mixture of isomeric epoxyacetates **11a** and **11b**, identified by comparison with authentic samples.[11] The $\Delta^{13(16)}$ isomer **12** was therefore easily isolated from the reaction mixture and was purified by Si gel chromatography (40%) and treated by KOH−EtOH, affording, after chromatographic purification, the alcohol **13** (95%). The latter compound was oxidized by pyridinium dichromate (PDC) in CH_2Cl_2 to the aldehyde **14** (87%) and then with $NaClO_2$−NaH_2PO_4 to the carboxylic acid **15** (94%). According to the previously described procedure,[12] **15** was converted by treatment with $(COCl)_2$−C_6H_6 into the corresponding chloride **16**, which was coupled, without purification, with (+)-1,2-*O*-isopropylidene-*sn*-glycerol to afford the acetonide **17** (85% from the acid **15**). Deprotection of **17** gave, after chromatographic purification, the monoglyceryl ester **18** (87%), which was acetylated with *N*-acetylimidazole (DBU, C_6H_6) to give the 3′-acetyl derivative **8** (64%), which showed MS and NMR data identical with those of natural **1**, but with the opposite $[\alpha]_D$ and CD profile.

Anisodorin 2 (**2**) proved to be the 2′-acetyl isomer of **1**, by analysis of both ^{1}H and ^{13}C NMR spectra, which differed significantly only in the shift values at C-2′ and C-3′ of the glyceryl moiety (Tables 1 and 2). All NMR resonances were assigned by comparison with those of **1**.

Anisodorin 3 (**3**) showed a molecular formula $C_{25}H_{42}O_6$, as deduced from its MS and ^{13}C NMR data, which implied the presence of an additional hydroxy group in the molecule. The 1,3-diacylation pattern of the glycerol was

Scheme 1[a]



[a] Reagents and conditions: (i) $POCl_3$, dry pyr., reflux (30 min), 88%; (ii) monoperphthalic acid, Et_2O, room temperature (2.5 h); (iii) 10% KOH/EtOH, reflux (2 h), 96%; (iv) PDC, CH_2Cl_2, room temperature (24 h), 84%; (v) $NaClO_2$, *t*-BuOH, 2-methyl-2-butene, NaH_2PO_4, room temperature (3.5 h), 92%; (vi) $(COCl)_2/C_6H_6$, Ar, room temperature (2 h), 45 °C (30 min); (vii) (+)-1,2-*O*-isopropyliden-*sn*-glycerol, NaH, CH_2Cl_2, 0 °C (15 min), room temperature (10 min), 85%; (viii) 0.006 M H_2SO_4/MeOH, Ar, room temperature (5 h), 87%; (ix) *N*-acetylimidazole, C_6H_6, DBU, room temperature (1 h), 64%.

Table 2. ^{13}C NMR Data[a,b] of Anisodorins 1−5 (**1**−**5**)

	δ ^{13}C m[c]				
carbon	**1**	**2**	**3**	**4**	**5**
1	40.0 t	40.0 t	40.1 t	40.0 t	39.9 t
2	18.5[d] t	18.5[f] t	18.6 t	18.5 t	18.1 t
3	42.0 t	42.0 t	42.1 t	42.0 t	42.0 t
4	33.3 s	n.d.[i]	33.3 s	n.d.	33.3 s
5	56.7 d	56.7 d	56.7 d	56.7 d	56.4 d
6	18.6[d] t	18.7[f] t	18.1 t	18.0 t	18.5[h] t
7	40.5 t	40.5 t	42.3 t	42.2 t	41.2 t
8	39.8 s	n.d.	38.7 s	n.d.	38.4 s
9	59.2 d	59.2 d	59.7 d	59.6 d	60.3 d
10	37.8 s	n.d.	37.7 s	n.d.	37.5 s
11	22.1 t	22.1 t	17.2 t	17.2 t	18.8[h] t
12	36.1 t	36.1 t	40.1 t	40.0 t	38.1 t
13	143.4 s	n.d.	70.4 s	n.d.	73.1 s
14	63.2 d	63.2 d	64.2 d	64.1 d	59.8 d
15	171.6 s	n.d.	175.2 s	n.d.	61.6 t
16	108.2 t	108.2 t	30.9 q	30.8 q	67.3 t
17	15.0 q	15.0 q	16.9 q	16.9 q	17.2 q
18	21.5 q	21.5 q	21.4 q	21.3 q	21.4 q
19	33.4 q	33.4 q	33.3 q	33.2 q	33.3 q
20	16.2 q	16.2 q	16.3 q	16.3 q	16.2 q
O*C*OCH$_3$-15					171.4 s
OCO*C*H$_3$-15					21.0 q
O*C*OCH$_3$-16					171.0 s
OCO*C*H$_3$-16					21.4 q
1′	64.6[e] t	61.6[g] t	64.9 t	62.0 t	
2′	68.3 d	72.4 d	68.3 d	72.1 d	
3′	65.3[e] t	61.4[g] t	65.2 t	61.7 t	
−O*C*OCH$_3$	171.0 s	n.d.	171.0 s	n.d.	
−OCO*C*H$_3$	20.8 q	n.d.	20.8 q	21.0 q	

[a] Bruker AMX 500 MHz, $CDCl_3$, chemical shifts (ppm) referred to *C*DCl_3 (δ 77.0). [b] Assignments made by HMQC and HMBC (*J* = 10 Hz). [c] By DEPT sequence. [d−h] Values with identical superscript may be reversed. [i] n.d. = not determined.

indicated by diagnostic signals at δ 4.11 (m, H-2′), 4.16 (m, H_2-3′), 4.18 (dd, *J* = 11.5, 4.3 Hz, H-1′a), and 4.26 (dd, *J* = 11.5, 4.0 Hz, H-1′b) in the ^1H NMR spectrum, and at δ 64.9 (t), 65.2 (t), and 68.3 (d) in the ^{13}C NMR spectrum. The proton spectrum displayed five singlet methyl signals at δ 0.80, 0.85, 0.87, 1.11, and 1.18, suggesting the presence of a saturated isocopalane skeleton containing a tertiary hydroxyl group at C-13, as supported by the deshielded chemical shift values of both C-13 (δ 70.4) and C-16 (δ ^1H 1.11, δ ^{13}C 30.9). Diagnostic correlations in the HMBC spectrum between H_3-16 (δ 1.11) and C-12 (δ 40.1), C-13 (δ 70.4) and C-14 (δ 64.2), and the OH (δ 3.59) and C-12 (δ 40.1) further confirmed these assignments. The relative stereochemistry at C-13 and C-14 was inferred by a series of NOE difference experiments. In particular, a strong NOE effect between H-14 (δ 2.15) and H_3-16 (δ 1.11) indicated a *cis*-orientation of H-14 and H_3-16. In addition, irradiation of the hydroxyl proton at δ 3.59 resulted in the enhancement of H_3-17 (δ 1.18), while irradiation of H-14 (δ 2.15) induced an enhancement of H-9 (δ 0.83), establishing a *cis*-relationship between H-14 and H-9 and between the hydroxyl group at C-13 and the methyl group at C-8. The equatorial orientation of the C-13 methyl group was further supported by comparison of the diagnostic ^{13}C NMR chemical shift value of H_3-16 (δ 30.9) with analogous data reported in the literature for the tertiary methyl at C-13 of related isocopalane diterpenes.[9,13] All NMR assignments were made by one- and two-dimensional experiments and are reported in Tables 1 and 2. The CD curve of anisodorin 3 (**3**) was identical to those of **1** and **6**, suggesting that they possessed the same absolute stereochemistry for the diterpenoid moiety. The absolute configuration of C-2′ of the

glyceryl fragment was suggested to be *S*, analogous to that of the other anisodorins.

Anisodorin 4 (**4**) displayed close spectral analogies with **3**, which revealed that the only difference was that the acetyl group was positioned at C-2′ of the glyceryl moiety rather than at C-3′. All proton and carbon resonances were assigned by NMR one- and two-dimensional experiments (Tables 1 and 2).

Anisodorin 5 (**5**) showed a molecular formula $C_{24}H_{40}O_5$, as deduced from both its MS and ^{13}C NMR spectra. Analysis of the NMR data of compound **5** revealed the absence of a glyceryl fragment in the molecule. The spectra showed two acetyl groups (δ_C 171.0, δ_H 2.10; δ_C 171.4, δ_H 2.06) both linked to methylenes [δ_C 67.3, δ_H 4.02 (d, J = 11.7 Hz) and 4.25 (br d, J = 11.7 Hz); δ_C 61.6, δ_H 4.20 (dd, J = 11.9, 6.0 Hz) and 4.52 (dd, J = 11.9, 3.6 Hz)] and four methyls linked to sp^3 quaternary carbons [δ_C 16.2, δ_H 0.81; δ_C 17.2, δ_H 0.89; δ_C 21.4, δ_H 0.81; δ_C 33.3, δ_H 0.86]. In addition, the presence of a tertiary hydroxyl group was revealed by both a singlet signal, exchangeable with D_2O, at δ 3.03 in the ^1H NMR spectrum, and a quaternary carbon at δ 73.1 in the ^{13}C NMR spectrum. These data strongly suggested a saturated isocopalane skeleton displaying an oxidized methyl at C-13, further linked to an OH group, and a $-CH_2OAc$ moiety in the place of the ester carboxyl function at C-14. This structural hypothesis was supported by diagnostic HMBC correlations between C-14 (δ 59.8) and H-15a (δ 4.20), −OH (δ 3.03) and H_3-17 (δ 0.89), and C-12 (δ 38.1) and H-16a (δ 4.02). The relative stereochemistry of the chiral centers of the molecule was established by a series of NOE difference experiments. In particular, a strong effect was observed among H_3-17 (δ 0.89), H_2-16 (δ 4.02 and δ 4.25), and H-15a (δ 4.20), supporting the same orientation for the methyl at C-8 and the two methylene groups at C-13 and C-14. In addition, irradiation of the proton signal at δ 1.70 (H-14) induced an enhancement of the proton signal at δ 0.96 (H-9), confirming the *cis*-relationship of H-9 and H-14. A weak negative NOE interaction among the −OH proton (δ 3.03) and H-9 and H-14 was also observed. All proton and carbon resonances were assigned by NMR one- and two-dimensional experiments (Tables 1 and 2). The absolute configuration of **5** is suggested to be the same as the other anisodorins, although the opposite stereochemistry, typical for metabolites from sponges belonging to the genus *Spongia*, cannot be excluded. Therefore, in contrast to the other anisodorins, which are most likely biosynthesized *de novo*, anisodorin 5 could be derived from a dietary source, although we were not able to detect traces of **5** in the digestive gland of the mollusk.

Experimental Section

General Experimental Procedures. Melting points were measured on a Kofler apparatus and are uncorrected. Optical rotations were measured in $CHCl_3$ on a JASCO DIP 370 digital polarimeter, and CD curves were recorded in EtOH solution on a JASCO 710 spectropolarimeter. The IR spectra were taken on a Bio-Rad FTS 7 spectrophotometer. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ on Bruker WM 500, Bruker AM 400, and Bruker WM 300 spectrometers, and chemical shifts are reported in parts per million, referenced to $CHCl_3$ as internal standard (δ 7.26 for proton and δ 77.0 for carbon). Mass spectra were recorded on a Carlo Erba TRIO 2000 VG instrument. Si gel chromatography was performed using commercial Merck Kieselgel 60 powder (70−230 mesh ASTM) and Merck precoated F_{254} plates. TLC plates were sprayed with 0.1% $Ce(SO_4)_2$ in 2N H_2SO_4 and heated at 80 °C for 5 min to detect the spots. HPLC purifications were conducted on a Waters liquid chromatograph equipped with a differential refractometer as detector.

The workup of the reaction mixtures in organic solvents involved exhaustive extraction with Et_2O and washing with H_2O, until neutral pH. Acidic solutions were washed further with saturated $NaHCO_3$ aqueous solution, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo.

Animal Material. *Anisodoris fontaini* (30 individuals, average size 4 cm) was collected along the Patagonian coast (southern Argentina) by snorkeling, at a depth of 1−5 m, during the months of November and December 1991 and January 1992. A voucher specimen representing these collections is available at Departamento de Biologia de Organismos y Sistemas, Universidad de Oviedo, Oviedo, Spain.

Extraction and Isolation. *A. fontaini* specimens (dry wt 33 g) were extracted with Me_2CO (3 × 30 mL) using ultrasonic vibration. After removal of the solvent, the animals were ground in a mortar and extracted again with Me_2CO (4 × 30 mL). Each Me_2CO extract was evaporated under reduced pressure, and the residue was partitioned between Et_2O and H_2O. The organic fractions of both ultrasonic and powder extracts were concentrated in vacuo giving 213 mg and 864 mg, respectively, of crude material, which was analyzed by TLC (petroleum ether−Et_2O, 1:1). A series of spots at R_f 0.45−0.30 were detected only in the ultrasonic extract, and this was chromatographed on a Si gel column, using petroleum ether with increasing amounts of Et_2O.

Fractions eluted with petroleum ether−Et_2O, 1:1 (63 mg), containing anisodorins, were submitted to HPLC purification [Spherisorb S5W, 3.9 mm (i.d.) × 30 cm; *n*-hexane−EtOAc 85:15, flow rate 2.0 mL/min], obtaining in order of t_R, anisodorin 1 (**1**, 1.4 mg), the already reported **6** (22.8 mg), anisodorin 2 (**2**, 1.0 mg), and the known **7** (9.1 mg). The combined fractions eluted with petroleum ether−Et_2O 3:7 (7.3 mg) also containing anisodorins, were purified in a similar manner by normal-phase HPLC [Spherisorb S5W, 3.9 mm (i.d.) × 30 cm; *n*-hexane−EtOAc 80:20, flow rate 1.5 mL/min], affording anisodorin 3 (**3**, 1.3 mg), anisodorin 4 (**4**, 2.0 mg), and anisodorin 5 (**5**, 1.5 mg).

Anisodorin 1 (1): 1.4 mg; $[\alpha]_D$ +2.1° (*c* 0.14, $CHCl_3$); CD $[\theta]_{225}$ (EtOH) +2827; IR (liquid film) ν_{max} 3468, 1742, 1238 cm^{-1}; ^1H NMR data in Table 1; ^{13}C NMR data in Table 2; EIMS *m*/*z* 420 ([M^+], 1), 405 (1), 402 (1), 286 ([M^+−acetyl glycerol], 34), 271 (6), 259 (3), 244 (5), 191 (81), 69 (100).

Anisodorin 2 (2): 1.0 mg; $[\alpha]_D$ −92.9° (*c* 0.10, $CHCl_3$);[14] CD $[\theta]_{218}$ (EtOH) +1424; IR (liquid film) ν_{max} 3452, 1742, 1238 cm^{-1}; ^1H NMR data in Table 1; ^{13}C NMR data in Table 2; EIMS *m*/*z* 420 ([M^+], 2), 405 (3), 402 (2), 286 ([M^+−acetyl glycerol], 78), 271 (19), 259 (8), 244 (13), 205 (21), 191 (100).

Anisodorin 3 (3): 1.3 mg; $[\alpha]_D$ +2.9° (*c* 0.13, $CHCl_3$); CD $[\theta]_{218}$ (EtOH) +7574; IR (liquid film) ν_{max} 3429, 1745, 1699 cm^{-1}; ^1H NMR data in Table 1; ^{13}C NMR data in Table 2; EIMS *m*/*z* 438 ([M^+], 1), 420 (6), 405 (7), 304 ([M^+−acetyl glycerol], 46), 289 (43), 191 (76), 176 (75), 158 (61), 117 (100).

Anisodorin 4 (4): 2.0 mg; $[\alpha]_D$ +17.9° (*c* 0.20, $CHCl_3$); CD $[\theta]_{218}$ (EtOH) +5928; IR (liquid film) ν_{max} 3421, 1709 cm^{-1}; ^1H NMR data in Table 1; ^{13}C NMR data in Table 2; EIMS *m*/*z* 438 ([M^+], 1), 420 (3), 405 (4), 347 (3), 304 ([M^+−acetyl glycerol], 18), 289 (19), 244 (17), 191 (41), 176 (51), 117 (100).

Anisodorin 5 (5): 1.5 mg; $[\alpha]_D$ −3.2° (*c* 0.15, $CHCl_3$); IR (liquid film) ν_{max} 3483, 1742 cm^{-1}; ^1H NMR data in Table 1; ^{13}C NMR data in Table 2; EIMS *m*/*z* 348 ([M^+−AcOH], 4), 330 (2), 305 (1), 275 ([M^+−AcOH−CH_2OAc], 25), 257 (9), 191 (28), 123 (47), 69 (100).

Synthesis of 8. (a) (14*R*)-*ent*-Isocopal-13(16)-en-15-acetate (11). $POCl_3$ (36.2 g, 236 mmol) was added dropwise, under Ar, to a 0 °C solution of **9**[11] (1.75 g, 5 mmol in 100 mL of dry pyridine). The reaction mixture was stirred, under an Ar atmosphere, at 0 °C for 30 min, at room temperature for 30 min, and then at reflux for 30 min. The usual workup afforded 1.61 g of a crude reaction product, which was purified on a Si gel column (petroleum ether−Et_2O 19:1), giving 1.47 g (88%) of a mixture of the $\Delta^{12(13)}$ (**11**) and the $\Delta^{13(16)}$ (**12**) isomeric acetates (3:2). The mixture (1.47 g, 4.4 mmol) was

dissolved in 5 mL of Et_2O, and 20 mL of an Et_2O solution of monoperphthalic acid (76 mmol) were added. The reaction mixture was stirred at room temperature for 2.5 h, washed with a 2% NaOH aqueous solution and H_2O, and finally dried.

The solvent was removed at reduced pressure, and the residue (1.51 g) was chromatographed on a Si gel column (petroleum ether−Et_2O gradient) to yield 588 mg (40%) of pure crystalline **12**: mp 98−99.5 °C (from petroleum ether); $[\alpha]_D$ −30.6° (*c* 0.25, $CHCl_3$); IR (liquid film) ν_{max} 1227, 1722 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 4.83 (1H, d, J = 1.2 Hz, H-16a), 4.50 (1H, d, J = 1.3 Hz, H-16b), 4.33 (1H, dd, J = 11.2, 3.6 Hz, H-15a), 4.17 (1H, dd, J = 11.2, 9.4 Hz, H-15b), 2.38 (1H, m, H-12a), 2.02 (3H, s, OAc), 1.80 (1H, dd, J = 9.4, 3.6 Hz, H-14), 0.86 (3H, s, CH_3-18), 0.81 (3H, s, CH_3-19), 0.80 (3H, s, CH_3-20), 0.75 (3H, s, CH_3-17); ^{13}C NMR (75.5 MHz) δ 171.4 (OAc), 146.8 (C-13), 106.8 (C-16), 61.5 (C-15), 59.8 (C-9), 56.4 (C-5), 55.1 (C-14), 42.0 (C-3), 40.6 (C-7), 40.1 (C-1), 39.3 (C-8), 37.8 (C-10), 37.5 (C-12), 33.3 (2C, C-18 and C-4), 22.7 (C-11), 21.4 (C-19), 21.1 (OAc), 18.9 (C-2 or C-6), 18.6 (C-6 or C-2), 16.3 (C-20), 16.1 (C-17); EIMS *m*/*z* 272 ([M^+−AcOH], 6), 256 (5), 207 (25), 191 (42), 149 (94), 69 (72), 57 (100). Further elution afforded a mixture (100 mg) of the isomeric epoxyacetates **11a** and **11b**, and finally pure **11b** (769 mg). Compounds **11a** and **11b** were identified by comparison with authentic samples.[11]

(b) (14*R*)-*ent*-Isocopal-13(16)-en-15-ol (13). A 10% KOH−EtOH solution (12 mL) was added to 556 mg (1.7 mmol) of **12** dissolved in 2 mL of EtOH. The mixture was refluxed for 2 h. At the end of this period, the reaction was stopped to give, after the usual workup, 501 mg of a crude reaction product, which was purified on a Si gel column (petroleum ether−Et_2O, 24:1) to yield 464 mg (96%) of pure crystalline alcohol **13**: mp 128−129.5 °C (from petroleum ether); $[\alpha]_D$ −11.2° (*c* 0.09, $CHCl_3$); IR (liquid film) ν_{max} 3398 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.92 (1H, br s, H-16a), 4.63 (1H, br s, H-16b), 3.80 (2H, m, H_2-15), 2.41 (1H, m, H-12a), 0.86 (3H, s, CH_3-18), 0.82 (3H, s, CH_3-19), 0.80 (3H, s, CH_3-20), 0.72 (3H, s, CH_3-17); ^{13}C NMR (75.5 MHz) δ 147.8 (C-13), 106.0 (C-16), 60.0 (C-9 or C-15), 59.6 (C-15 or C-9), 58.7 (C-14), 56.5 (C-5), 42.0 (C-3), 40.7 (C-7), 40.1 (C-1), 39.7 (C-8), 38.3 (C-10), 37.8 (C-12), 33.3 (2C, C-18 and C-4), 23.0 (C-11), 21.4 (C-19), 19.0 (C-2 or C-6), 18.6 (C-6 or C-2), 17.8 (C-17), 16.3 (C-20); EIMS *m*/*z* 290 ([M^+], 16), 275 (19), 272 (7), 259 (28), 245 (12), 204 (22), 191 (100), 177 (84); HREIMS *m*/*z* 290.2602 (calcd for $C_{20}H_{34}O$, 290.2610).

(c) (14*R*)-*ent*-Isocopal-13(16)-en-15-al (14). PDC (750 mg) was added to a solution of alcohol **13** (303 mg, 1.04 mmol) in dry CH_2Cl_2 (40 mL). The reaction mixture was stirred at room temperature for 24 h, and then filtered on Si gel to give 295 mg of a crude product, which was chromatographed on a Si gel column (petroleum ether−Et_2O, 99:1) to give 252 mg (84%) of a crystalline aldehyde (**14**): mp 94−95 °C (from petroleum ether); $[\alpha]_D$ +39.9° (*c* 0.09, $CHCl_3$); IR (liquid film) ν_{max} 1722 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 9.89 (1H, d, J = 4.9 Hz, H-15), 4.90 (1H, br s, H-16a), 4.50 (1H, br s, H-16b), 2.43 (1H, m, H-14), 2.41 (1H, m, H-12a), 2.07 (1H, m, H-12b), 1.15 (3H, s, CH_3-17), 0.87 (3H, s, CH_3-18), 0.85 (3H, s, CH_3-20), 0.81 (3H, s, CH_3-19); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 205.8 (C-15), 145.0 (C-13), 108.9 (C-16), 68.2 (C-14), 58.7 (C-9), 56.6 (C-5), 41.9 (C-3), 41.2 (C-7), 39.9 (C-1), 39.5 (C-8), 37.9 (C-10), 36.6 (C-12), 33.3 (2C, C-18 and C-4), 21.9 (C-11), 21.5 (C-19), 18.5 (C-2 or C-6), 18.5 (C-6 or C-2), 17.0 (C-20 or C-17), 16.4 (C-17 or C-20); EIMS *m*/*z* 288 ([M^+], 4), 271 (3), 258 (2), 207 (6), 191 (28), 177 (9), 149 (23), 109 (68), 69 (96), 55 (100).

(d) (14*R*)-*ent*-Isocopal-13(16)-en-15-oic acid (15). According to the procedure described by Balkrishna et al.,[15] 426 mg of $NaClO_2$ and 496 mg of NaH_2PO_4, dissolved in 3.2 mL of H_2O, and 1.9 mL of 2-methyl-2-butene, were added to a solution of aldehyde **14** (230 mg, 0.8 mmol) in *t*-BuOH (8 mL). The reaction mixture was stirred at room temperature for 3.5 h. The usual workup afforded a crude residue (239 mg) that was purified on a Si gel column (petroleum ether−Et_2O, 93:7) to obtain crystalline **15** (228 mg, 94%): mp 222−223 °C (from petroleum ether−Et_2O); $[\alpha]_D$ +1.2° (*c* 0.12, $CHCl_3$); IR (liquid film) ν_{max} 1699 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.85 (1H, br s, H-16a), 4.78 (1H, br s, H-16b), 2.83 (1H, s, H-14), 2.41 (1H, m, H-12a), 2.05 (1H, m, H-12b), 1.05 (3H, s, CH_3-17), 0.86 (3H, s, CH_3-18), 0.85 (3H, s, CH_3-20), 0.81 (3H, s, CH_3-19); ^{13}C NMR (75.5 MHz, $CDCl_3$,) δ 177.2 (C-15), 143.1 (C-13), 108.3 (C-16), 63.1 (C-14), 59.2 (C-9), 56.7 (C-5), 42.0 (C-3), 40.4 (C-7), 40.0 (C-1), 39.6 (C-8), 37.8 (C-10), 36.1 (C-12), 33.4 (C-18), 33.3 (C-4), 22.1 (C-11), 21.5 (C-19), 18.6 (C-6 or C-2), 18.5 (C-2 or C-6), 16.2 (C-20), 14.9 (C-17); EIMS *m*/*z* 304 ([M^+], 3), 289 (4), 244 (2), 205 (10), 191 (69), 123 (41), 109 (35), 69 (100).

(e) (14*R*)-*ent*-Isocopal-13(16)-en-15-oyl chloride (16). A solution of $(COCl)_2$ (0.2 mL, 2 mmol) in dry C_6H_6 (1.2 mL) was added to a solution of acid **15** (120 mg, 0.4 mmol) in dry C_6H_6 (1.2 mL), under an Ar atmosphere. The reaction mixture was stirred at room temperature for 2 h and then at 45 °C for 30 min. At the end of this period, the solvent and the unreacted oxalyl chloride were removed in vacuo, to give 127 mg of crude **16** [IR (liquid film) ν_{max} 1705 cm^{-1}], which was used in the next step without purification.

(f) 3-(14*R*)-*ent*-Isocopal-13(16)-en-15-oyl-1,2-*O*-isopropylidene-*sn*-glycerol (17). A suspension of NaH (45 mg, 1.5 mmol, 80% dispersed in mineral oil) and 0.1 mL of dry pyridine were added to a solution of (+)-1,2-*O*-isopropylidene-*sn*-glycerol (186 mg, 1.4 mmol) in anhydrous CH_2Cl_2 (6.8 mL), at 0 °C, under an Ar atmosphere. The mixture was stirred for 10 min and then crude **16** (127 mg, 0.4 mmol) was added. The reaction mixture was stirred at 0 °C for 20 min and at room temperature for 10 min. After the usual workup, the crude residue was purified on a Si gel column (petroleum ether−Et_2O, 93:7) to give 141 mg (85%) of acetonide **17**: oil; $[\alpha]_D$ −11.9° (*c* 0.17, $CHCl_3$); IR (liquid film) ν_{max} 1741 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.81 (1H, br s, H-16a), 4.66 (1H, br s, H-16b), 4.29 (1H, app. quintet, J = 5.8 Hz, H-2′), 4.10 (2H, m, H_2-3′), 4.06 (1H, m, H-1′a), 3.74 (1H, dd, J = 8.4, 6.1 Hz, H-1′b), 2.83 (1H, s, H-14), 2.39 (1H, br dd, J = 13.4, 2.3 Hz, H-12a), 2.02 (1H, ddd, J = 13.2, 13.0, 5.0 Hz, H-12b), 1.05 (3H, s, CH_3-17), 0.85 (3H, s, CH_3-18), 0.84 (3H, s, CH_3-20), 0.80 (3H, s, CH_3-19); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 171.2 (C-15), 143.4 (C-13), 109.7 (C quaternary acetonide), 108.1 (C-16), 73.6 (C-2′), 66.7 (C-3′), 64.0 (C-1′ or C-14), 63.1 (C-14 or C-1′), 59.2 (C-9), 56.7 (C-5), 42.0 (C-3), 40.4 (C-7), 40.0 (C-1), 39.7 (C-8), 37.8 (C-10), 36.1 (C-12), 33.4 (C-18), 33.3 (C-4), 26.7 (CH_3 acetonide), 25.4 (CH_3 acetonide), 22.1 (C-11), 21.5 (C-19), 18.6 (C-6 or C-2), 18.5 (C-2 or C-6), 16.2 (C-20), 15.0 (C-17); EIMS *m*/*z* 418 ([M^+], 1), 403 (27), 360 (22), 345 (14), 286 (41), 223 (29), 191 (100), 169 (64), 156 (81).

(g) 3-(14*R*)-*ent*-Isocopal-13(16)-en-15-oyl-*sn*-glycerol (18). Compound **17** (120 mg, 0.3 mmol) was dissolved in 0.6 mL of MeOH and then 5.2 mL of H_2SO_4−MeOH (0.006 M) were added at room temperature in an Ar atmosphere. The mixture was stirred at room temperature for 5 h. The usual work up gave 107 mg of a crude residue, which was purified on a Si gel column (petroleum ether−Et_2O, 1:1) giving 94 mg (87%) of a crystalline monoacylglycerol (**18**): mp 131−132 °C (from petroleum ether); $[\alpha]_D$ −4.7° (*c* 0.71, $CHCl_3$); IR (liquid film) ν_{max} 1735, 3392 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.82 (1H, d, J = 0.5 Hz, H-16a), 4.65 (1H, d, J = 0.7 Hz, H-16b), 4.20 (1H, dd, J = 11.6, 4.7 Hz, H-3′a), 4.12 (1H, dd, J = 11.6, 6.0 Hz, H-3′b), 3.92 (1H, m, H-2′), 3.69 (1H, dd, J = 11.5, 4.0 Hz, H-1′a), 3.60 (1H, dd, J = 11.5, 5.8 Hz, H-1′b), 2.83 (1H, s, H-14), 2.40 (1H, m, H-12a), 2.03 (1H, m, H-12b), 1.06 (3H, s, CH_3-17), 0.85 (3H, s, CH_3-18), 0.84 (3H, s, CH_3-20), 0.80 (3H, s, CH_3-19); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 172.0 (C-15), 143.5 (C-13), 108.1 (C-16), 70.3 (C-2′), 64.7 (C-3′), 63.4 (C-1′), 63.2 (C-14), 59.2 (C-9), 56.7 (C-5), 42.0 (C-3), 40.5 (C-7), 40.0 (C-1), 39.8 (C-8), 37.8 (C-10), 36.1 (C-12), 33.4 (C-18), 33.3 (C-4), 22.1 (C-11), 21.5 (C-19), 18.6 (C-6 or C-2), 18.5 (C-2 or C-6), 16.2 (C-20), 15.0 (C-17); EIMS *m*/*z* 378 ([M^+], 3), 363 (4), 286 (42), 271 (11), 244 (10), 205 (14), 191 (100), 123 (59), 95 (78); HREIMS *m*/*z* 378.2780 (calcd for $C_{23}H_{38}O_4$, 378.2770).

(h) 3-(14*R*)-*ent*-Isocopal-13(16)-en-15-oyl-1-acetyl-*sn*-glycerol (8). DBU (1,8-diazabicyclo-[5.4.0]-undec-7-ene) (3.1 mg, 0.03 mmol) and 13.6 mg (0.12 mmol) of *N*-acetylimidazole were added to 30 mg (0.08 mmol) of the monoacylglycerol **18**, dissolved in 0.6 mL of dry C_6H_6. The mixture was stirred in an Ar atmosphere at room temperature for 1 h, the reaction was stopped by addition of 1 mL of H_2O and, after the usual

workup, the solvent was removed in vacuo. The crude residue (31.4 mg) was chromatographed on a Si gel column (petroleum ether−Et_2O, 93:7) affording 19.5 mg (64%) of the 1,3-diacylglycerol **8**: oil; $[\alpha]_D$ −6.9° (*c* 0.55, $CHCl_3$); CD $[\theta]_{225}$ (EtOH) −3172; IR (liquid film) ν_{max} 1228, 1742, 3444 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 4.82 (1H, br s, H-16a), 4.66 (1H, br s, H-16b), 4.14 (4H, m, H_2-1′ and H_2-3′), 4.10 (1H, m, H-2′), 2.84 (1H, s, H-14), 2.40 (1H, m, H-12a), 2.10 (3H, s, OAc), 2.03 (1H, m, H-12b), 1.06 (3H, s, CH_3-17), 0.86 (3H, s, CH_3-18), 0.84 (3H, s, CH_3-20), 0.81 (3H, s, CH_3-19); ^{13}C NMR (75.5 MHz, $CDCl_3$,) δ 171.6 (C-15), 171.0 (OAc), 143.4 (C-13), 108.2 (C-16), 68.3 (C-2′), 65.2 (C-3′), 64.6 (C-1′), 63.2 (C-14), 59.2 (C-9), 56.7 (C-5), 42.0 (C-3), 40.5 (C-7), 40.0 (C-1), 39.8 (C-8), 37.8 (C-10), 36.1 (C-12), 33.4 (C-18), 33.3 (C-4), 22.1 (C-11), 21.5 (C-19), 20.8 (OAc), 18.6 (C-6 or C-2), 18.5 (C-2 or C-6), 16.2 (C-20), 15.0 (C-17); EIMS *m/z* 420 ([M^+], 1), 405 (3), 347 (3), 286 (75), 271 (19), 244 (15), 205 (19), 191 (100), 123 (87), 117 (91).

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Supporting Information Available: ^1H NMR spectra of **1**−**5** and ^{13}C NMR spectra of **1**, **3**, and **5** (8 pages). Ordering information is given on any current masthead page.

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(14) The optical activity of **2** was measured many times giving the reported mean value, which is different in its sign from that of the corresponding 1,3-derivative (**1**). Most likely, this is due to the presence of an impurity that affected the $[\alpha]_D$ value of **2**. However, the absolute stereochemistry of **2** is the same as **1**, as supported by both the similar CD profile and the transformation in $CHCl_3$ solution of **2** in **1**.
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