# Archidorin: A New Ichthyotoxic Diacylglycerol from the Atlantic Dorid Nudibranch Archidoris tuberculata

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# ARCHIDORIN: A NEW ICHTHYOTOXIC DIACYLGLYCEROL FROM THE ATLANTIC DORID NUDIBRANCH *ARCHIDORIS TUBERCULATA*[1]

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ABSTRACT.—An unusual diacylglycerol, archidorin [**1**], has been isolated from the mantle of the mollusk *Archidoris tuberculata*. Glycerol is esterified with tiglic acid and with a diterpenoid clerodane acid at positions 1-*sn* and 2-*sn*, respectively. Absolute stereochemistry of the glycerol residue is assigned by chemical correlation with 1,2-*O*-isopropylidene-*sn*-glycerol.

Opisthobranchs are marine mollusks which have elaborated some alternative defensive strategies that include the use of chemicals, because they are poorly protected by their shells (1–3). In particular, some naked dorid nudibranchs are protected against predators by the presence of ichthyotoxic acylglycerols in their mantles. Glycerols esterified with diterpenoid acids were first found in the British Columbian *Archidoris montereyensis* (4) and *Archidoris odhneri* (5), then in the Mediterranean *Doris verrucosa* (6) and in the Antarctic *Austrodoris kerguelensis* (7) and, finally in the Atlantic *Archidoris tuberculata* Müller (Nudibranchia, Archidoridídae) (8) (from North Spain) and *Archidoris carvi* (8) (from Argentina). We now report the structural characterization of a very unusual diacylglycerol, named archidorin [**1**], isolated from the skin extract of *A. tuberculata*.

Recently (8), analysis of the secondary metabolites from the mantle of *A. tuberculata* led to the characterization of two diacylglycerols, **2** and **3**, previously isolated from *A. montereyensis* (4,5), along with a minor component **1** in an amount too poor to allow complete structural work. A second collection of *A. tuberculata* (70 specimens) was carefully dissected. The $Et_2O$-soluble fraction (156 mg) from the $Me_2CO$ extract of the mantles was purified by cc ($SiO_2$, petroleum ether/$Et_2O$ gradient). The fraction (10 mg) containing **1** was further purified by hplc, yielding 4 mg of an optically active ($[\alpha]^{25}D$ $+12.1°$; $CHCl_3$, $c=0.3$) pure compound, named archidorin [**1**], $C_{28}H_{46}O_5$ deduced by hreims on the molecular peak at *m/z* 462.

The $^1H$-nmr spectrum of **1** displayed signals in accordance with a glycerol esterified at positions 1 and 2. The glycerol protons at δ 5.10 (1H, m), δ 4.32 (2H, m), and δ 3.73 (2H, m) shifted after acetylation to δ 5.33 (1H, m), 4.30 (2H, m), 4.22 (1H, dd, $J=6$ and 12 Hz), and 4.17 (1H, dd, $J=6$ and 12 Hz). Selective decoupling at δ 5.33 transformed the other resonances in two AB systems. The acyl residue of tiglic acid was suggested by a broad downfield quartet (H-3″, $J=6.5$ Hz) at δ 6.88 exhibiting direct and allylic couplings with the protons of two vinyl methyls resonating at δ 1.80 (d, $J=6.5$ Hz) and 1.83 (bs), respectively. An ir band at 1717 $cm^{-1}$, a uv max at 210 nm, and a mass fragment at *m/z* 362 [M−tiglic

[1]Independently, Prof. G. Sodano (University of Salerno) started in June 1992 to investigate *Archidoris pseudoargus* (=*A. tuberculata*).

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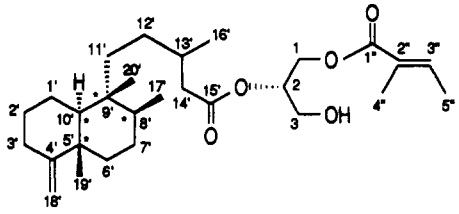
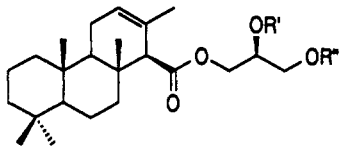
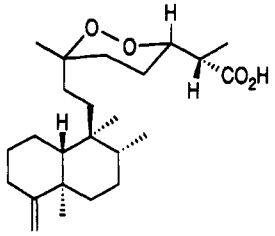


**1** (*relative stereochemistry)



**2** R′=H, R″=Ac
**3** R′=Ac, R″=H



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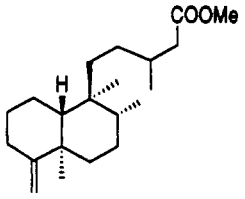

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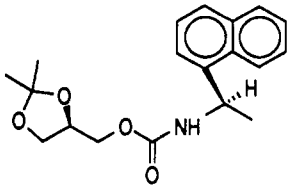
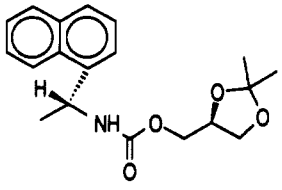


**6**



**7**

acid]$^+$ further supported the suggested partial structure. The $^{13}$C-nmr chemical shifts of C-4″ (δ 12.01) and C-5″ (δ 14.45) confirmed the cis relationship between the two methyls. The clerodane skeleton of the second acyl residue was suggested by the ir absorption at 1744 cm$^{-1}$, by the $^1$H-nmr multiplicity of the methyls (2 singlets and 2 doublets), and by some diagnostic ms fragments at *m/z* 289 (cleavage of the ester linkage), 191 [M−side chain]$^+$, and 95 [$C_7H_{11}$]$^+$. All the $^1$H- and $^{13}$C-nmr resonances (Table 1) of the diterpenoid were assigned by an extensive nmr study ($^1$H-$^1$H spin decouplings, $^1$H-$^1$H COSY, direct and long-range $^1$H-$^{13}$C HETCOR, HOHAHA) and by comparison with model compounds (9,10). In particular, all $^{13}$C-nmr resonances of the bicyclic skeleton were almost identical to those of the corresponding carbons of the bicyclic norsesterterpene sigmosceptrellin A [**4**] (9,10). However, the $^{13}$C-nmr δ values of C-2′ (28.67) and C-7′ (27.49), now assigned on the basis of HOHAHA and $^1$H-$^{13}$C HETCOR experiments, were reversed in comparison with the previous assignments. The $^{13}$C-nmr chemical shifts of C-10′, C-19′, C-17′, and C-20′ were diagnostic to assign to archidorin [**1**] the same relative stereochemistry as **4** at chiral centers 5′, 8′, 9′, and 10′. The nature of the side chain was suggested by $^1$H-$^1$H COSY and HOHAHA experiments, which connected all the protons at carbons 11′, 12′, 13′, 14′, and 16′, and by selective $^1$H-$^1$H decoupling at δ 1.82 (H-13′) which simultanenously collapsed the signals assigned to the protons at C-16′ (δ 0.92, 3H, d, $J$=6.6 Hz) and at C-14′ (δ 2.36, dd, $J$=14.9 and 5.7 Hz; δ 2.11, dd, $J$=14.9 and 8.5). The stereochemistry at C-13′ is undetermined. The two acyl residues were correctly linked to glycerol by an HMBC experiment ($J$=10 Hz). The resonance for C-15′ (δ 172.89) displayed diagnostic long-range correlations

TABLE 1. $^1$H- and $^{13}$C-nmr Data of Archidorin [1].[a]

| Position | δ $^{13}$C[b] | multiplicity[c] | δ $^1$H[b] |
|---|---|---|---|
| 1 | 62.13 | t | 4.32 |
| 2 | 72.20 | d | 5.10 |
| 3 | 61.51 | t | 3.73 |
| 1′ | 21.67 | t | 1.42–1.47 |
| 2′ | 28.67 | t | 1.25–1.86 |
| 3′ | 33.08 | t | 2.08–2.27 |
| 4′ | 160.80 | s | — |
| 5′ | 40.02 | s | — |
| 6′ | 37.33 | t | 1.42–1.57 |
| 7′ | 27.49 | t | 1.42–1.47 |
| 8′ | 36.57 | d | 1.42 |
| 9′ | 39.12 | s | — |
| 10′ | 48.60 | d | 1.05 |
| 11′ | 35.27 | t | 1.25 |
| 12′ | 29.48 | t | 1.05 |
| 13′ | 31.06 | d | 1.82 |
| 14′ | 41.68 | t | 2.11–2.36 |
| 15′ | 172.89 | s | — |
| 16′ | 19.81 | q | 0.92 |
| 17′ | 16.00 | q | 0.77 |
| 18′ | 102.39 | t | 4.49 |
| 19′ | 20.84 | q | 1.03 |
| 20′ | 18.26 | q | 0.71 |
| 1″ | 167.92 | s | — |
| 2″ | 128.02 | s | — |
| 3″ | 138.42 | d | 6.88 |
| 4″ | 12.01 | q | 1.83 |
| 5″ | 14.45 | q | 1.80 |

[a]Bruker AMX-500 spectrometer; $CDCl_3$; chemical shifts referred to $CHCl_3$ at 7.26 ppm and to $CDCl_3$ at 77.00 ppm.

[b]Assignments were aided by $^1$H-$^1$H COSY, HOHAHA, $^1$H-$^{13}$C HETCOR, $^1$H-$^{13}$C HMBC.

[c]By DEPT sequence.

with the protons at C-14′ and C-2, and analogously C-1″ (δ 167.92) was connected with the protons at C-4″, C-3″, and C-1.

In order to clarify some stereochemical details, **1** was treated with (*R*)-(−)-1-(1-naphthyl)-ethyl isocyanate, following the same procedure previously (11) adopted to determine the absolute stereochemistry of umbraculumins A and C. The urethane was isolated by $SiO_2$ chromatography and submitted to methanolysis (MeOH, $Na_2CO_3$), yielding mainly two products: a clerodane methyl ester with $^1$H-nmr spectrum (see Experimental) in accordance with that reported for the methyl ester of *ent*-clerod-4(18)-en-15-oic acid [**5**] (12) and a glyceryl urethane which by treatment with 2,2-dimethoxypropane yielded a mixture of **6** and **7**, identified by comparative hplc with standard compounds, in a ratio of 2:1. On the basis of this evidence, archidorin [**1**] is a 1,2-*sn*-diacyl-glycerol and co-occurs with the 2,3-*sn*-isomer that might derive by the easy migration of the tiglic acyl from position 1 to position 3 during the workup. A careful reanalysis of the $^1$H-nmr signals of the glycerol protons of **1**, and in particular of the ABX system at δ 4.32, revealed small amounts of a slightly downshifted (2.7 Hz) analogue system.

The absolute stereochemistry of the clerodane skeleton remains undetermined, even though the $^1$H-nmr spec-

trum of the clerodane methyl ester obtained from methanolysis of **1** is identical to that of **5** and the $[\alpha]_D$ of both are positive but with different absolute values: $[\alpha]^{25}_D$ +9.5°, $CHCl_3$, $c$=0.2; lit. (12) $[\alpha]^{24}_D$ + 32°, $CHCl_3$, $c$=0.93. However, related structures with assigned configurations are lacking in the literature (13). In fact, many incorrect stereochemical assignments are reported for clerodane skeletons and also for cyclic terpenoids displaying an exomethylene moiety at C-4 (14). On the basis of the co-occurrence of *ent*-isocopalane diterpenoids (**2**, **3**) in the same mollusk, we prefer an absolute stereochemistry with the methyls C-19′ and C-20′ β-oriented.

The ecological role of archidorin [**1**] may be linked to the defense of *A. tuberculata*, as supported by its high toxicity (toxic at 1 ppm) in the mosquito fish *Gambusia affinis* bioassay (15,16). Even though clerodane terpenoids have recently been found in a marine sponge (17), archidorin [**1**], analogously with **2** and **3** in *A. montereyensis* (5), is most likely biosynthesized de novo by *A. tuberculata*. However, very low amounts of radioactivity were recovered in archidorin [**1**] from preliminary in vivo experiments with labeled mevalonic acid.

## EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The ir spectra were recorded with a Nicolet FT 5DxB spectrometer. Optical rotations were measured on a Jasco DIP 370 polarimeter. The uv spectra were obtained on a Varian DMS 90 spectrophotometer. Hplc purifications were performed on a Waters chromatograph equipped with a differential refractometer and an uv detectors. $^1$H- and $^{13}$C-nmr spectra were recorded on a Bruker AM 500 spectrometer; chemical shifts are referred to $CHCl_3$ as internal standard (δ 7.26 for proton and δ 77.0 for carbon). Mass spectra were obtained on MS50 Kratos and TRIO 2000 VG instruments.

BIOLOGICAL MATERIALS.—*A. tuberculata* (70 specimens) were collected by SCUBA-diving at Ria del Eo (North Spain) in March 1992 and authenticated by J. Ortea. Voucher specimens are available at the Departamento de Biología de Organismos y Sistemas, Universidad de Oviedo.

EXTRACTION AND ISOLATION OF ARCHIDORIN [**1**].—Individuals (70 specimens) were carefully dissected in the mantle and digestive gland. The mantles were separately extracted with $Me_2CO$ at room temperature. The filtered $Me_2CO$ solution was concentrated under reduced pressure and then, after dilution with $H_2O$, extracted with $Et_2O$. The $Et_2O$-soluble fraction (156 mg) was chromatographed on a $SiO_2$ column using a petroleum ether/$Et_2O$ gradient. The fraction (10 mg) containing archidorin [**1**] ($R_f$ 0.4; $SiO_2$ tlc, petroleum ether-$Et_2O$ (1:1)] was further purified by hplc (Spherisorb 5 SIL; *n*-hexane–EtOAc (85:15)] yielding 4 mg of pure **1**.

*Archidorin* [**1**].—Amorphous powder: $[\alpha]^{25}_D$ 12.1° ($CHCl_3$, $c$=0.3); ir ν max (liquid film) 1739, 1717 $cm^{-1}$; uv λ max (MeOH) 210 (ε 12,300) nm; $^1$H and $^{13}$C nmr see Table 1; hreims *m/z* $[M]^+$ 462.3360 ($C_{28}H_{46}O_5$ requires 462.3345); eims *m/z* (%) $[M]^+$ 462 (0.5), $[M-18]^+$ 444 (1.4), 419 (2.1), $[M-C_5H_7O]^+$ 379 (4.2), $[M-C_5H_8O_2]^+$ 362 (1.5), 289 (1), 191 (51), 95 (73), 83 (100).

*Reaction of* **1** *with* (R)-(−)-1-(1-naphthyl)-ethyl isocyanate.—Compound **1** (2.7 mg) was dissolved in 0.5 ml of toluene, and 100 μl of (*R*)-(−)-1-(1-naphthyl)-ethyl isocyanate were added under $N_2$. The mixture was kept at 80° for 48 h, following the literature procedure (11). The urethane derivative was purified by a $SiO_2$ cc using $CHCl_3$ as eluent.

*Methanolysis of the urethane derivative.*—The urethane above obtained (3.5 mg) was dissolved in anhydrous MeOH (1 ml), and an excess of $Na_2CO_3$ was added. The solution was stirred at room temperature for 12 h, filtered, and the solvent evaporated. The residue was chromatographed on a $SiO_2$ column using first a petroleum ether/$Et_2O$ gradient and then a $CHCl_3$/MeOH gradient, obtaining two compounds. The clerodane methyl ester (2 mg): $[\alpha]^{25}_D$ +9.5° ($CHCl_3$, $c$=0.2); ms *m/z* (%) $[M]^+$ 320 (7), 305 (1), 289 (1), 191 (60), 95 (85), 83 (100); $^1$H nmr ($CDCl_3$) δ 4.49 (s, H-18), 3.65 (s, -OMe), 2.28 (m, $H_a$-3 and $H_a$-14), 2.09 (m, $H_b$-3 and $H_b$-14), 1.85 (m, $H_a$-2), 1.81 (m, H-13), 1.03 (s, H-19), 0.91 (d, $J$=6.6 Hz, H-16), 0.78 (d, $J$=6.3 Hz, H-17), 0.71 (s, H-20). The glyceryl urethane (1 mg) obtained was identified by comparison of tlc [$R_f$ 0.5, $CHCl_3$-MeOH (9:1)] and $^1$H-nmr spectrum with those of standard compounds prepared from 1,2- and 2,3-dipalmitoyl-*sn*-glycerol (11).

*Reaction of the glyceryl urethane with 2,2-dimethoxypropane.*—The glyceryl urethane obtained previously (1 mg) was dissolved in 2,2-dimethoxypropane (1 ml), and a catalytic amount of *p*-TsOH was added. The reaction mixture was stirred at room temperature for 24 h. $Na_2CO_3$ was

added, and the solvent was partially removed under $N_2$ flow. The residual solution was chromatographed on a $SiO_2$ column (petroleum ether/$Et_2O$ gradient) affording a mixture of **6** and **7**, which was compared with standard compounds by hplc (two Spherisorb 5 SIL columns connected in series, using as eluent *n*-hexane-iPrOH (99:1). Standards were prepared by reaction (75°, 18 h) of commercially available (Sigma) 1,2- and 2,3-*O*-isopropylidene-*sn*-glycerol with (*R*)-(−)-1-(1-naphthyl)-ethyl isocyanate. [The commercial samples were found contaminated each one by the other enantiomer, after transformation in the corresponding urethanes **6** and **7**. Compound **6** contained 15% of **7**, while **7** contained 32% of **6**, Pure **6** and **7** were obtained by preparative hplc.]

ICHTHYOTOXICITY TESTS.—The toxicity tests were conducted on the mosquito fish *G. affinis*, following the literature procedure (15,16).

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