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TETRAHEDRON: *ASYMMETRY*

# Absolute configuration of diterpenoid diacylglycerols from the Antarctic nudibranch *Austrodoris kerguelenensis*

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**Abstract**

The *R* absolute configuration at C-2′ of the glyceryl moiety of the natural diterpenoid 1,3-glyceryl esters **1** and **2** has been established by applying the modified Mosher method. These esters have been isolated, together with the corresponding 1,2-derivatives (**3** and **4**), from different collections of the Antarctic dorid nudibranch *Austrodoris kerguelenensis*. Surprisingly, such a configuration is opposite to that of all marine terpenoid diacylglycerols so far reported. Compounds **1** and **3** are new natural products closely related to austrodorin **5**, already described from the same species. 

Marine dorid nudibranchs belonging to the genera *Doris*, *Anisodoris*, *Archidoris* and *Austrodoris* are known to contain terpenoid glyceryl esters in their mantle.[1,2] These molecules, which are supposed to be involved in the chemical defensive mechanisms of the shell-less mollusc,[3,4] are potent activators of protein kinase C and very active in a regenerative test with the fresh water hydrozoan *Hydra vulgaris*.[5] In the course of our study on dorid nudibranchs, we have characterized a series of ichthyotoxic 1,3-*sn* and 1,2-*sn* diterpenoid diacylglycerols from several dorid species.[6–11] In order to determine the absolute stereochemistry of the diacylglycerols isolated, we have also performed synthetic strategies to obtain optically active terpenoid glyceryl esters.[12–14] All natural terpenoid diacylglycerols showed the *S* stereochemistry at C-2′ of the glyceryl moiety.

Herein, we report a stereochemical study on the 1,3-diacyl glyceryl esters **1** and **2**, which have been isolated from two different collections of the Antarctic nudibranch *Austrodoris kerguelenensis*, Bergh 1884, respectively, along with the corresponding 1,2-derivatives **3** and **4**. The new metabolites **1** and **3** were closely related to austrodorin **5**, previously found in a population of the same mollusc from

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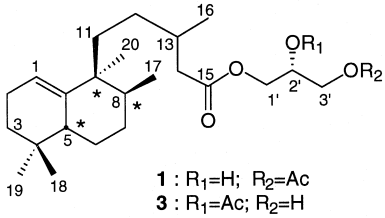
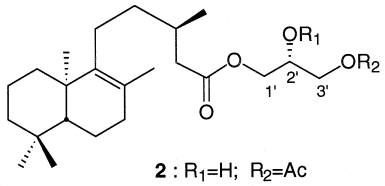
Tethys Bay,[9] whereas diacylglycerols **2** and **4** are known compounds, previously reported from an *A. kerguelenensis* specimen collected at McMurdo Sound.[15]



**1** : $R_1$=H; $R_2$=Ac
**3** : $R_1$=Ac; $R_2$=H
**5** : $R_1$=H; $R_2$=H
**6** : $R_1$=Ac; $R_2$=Ac
**7** : $R_1$=*S*-MTPA; $R_2$=Ac
**8** : $R_1$=*R*-MTPA; $R_2$=Ac



**2** : $R_1$=H; $R_2$=Ac
**4** : $R_1$=Ac; $R_2$=H
**9** : $R_1$=*S*-MTPA; $R_2$=Ac
**10** : $R_1$=*R*-MTPA; $R_2$=Ac

* = relative stereochemistry

Two specimens (13 cm length) of *A. kerguelenensis* were collected by trawling, South of Livingston Island (South Shetland Islands, Antarctica), within the framework of a Spanish expedition,[16] during January and February 1995. The specimens and mucus secreted by the molluscs were frozen immediately. Later, the animals were transferred to ICMIB and dissected into mantle and internal organs, which were separately extracted with acetone. Extracts were evaporated and the aqueous residue was treated with $Et_2O$. The mucus was extracted directly with $Et_2O$. A comparative TLC analysis (Si gel, petr. ether:$Et_2O$, 3:7) of $Et_2O$ soluble fractions of the different parts showed that the mucus and the mantle contained a series of metabolites ($R_f$ 0.5–0.1), which were absent in the remaining body sections. Therefore, $Et_2O$ extracts of mucus and mantle were combined (330 mg) and submitted to silica gel column chromatography, using a petroleum ether/$Et_2O$ gradient as eluent. The fraction eluted by petr. ether:$Et_2O$, 1:1 (70 mg), containing the more abundant metabolites at $R_f$ 0.4–0.5 (petr. ether:$Et_2O$, 3:7), was purified by normal-phase HPLC (*n*-hexane:isopropanol, 99:1) to give the 1,3 derivative **1**[17] (4.4 mg) and the main 1,2-derivative **3**[17] (22.4 mg). The more polar fraction eluted by $Et_2O$ (100 mg), containing metabolites at $R_f$ 0.1–0.3 (petr. ether:$Et_2O$, 3:7), was revealed, by preliminary NMR analysis, to contain a mixture of minor 2-monoacylglycerols, which has not been investigated further yet.

NMR data of compounds **1** and **3** immediately revealed strong similarities with those of austrodorin **5**, previously isolated from the same species.[9] In particular, **1** and **3** displayed a structure containing the same halimane diterpenoid acid linked to C-1′ of a glyceryl moiety, further esterified by an acetyl group at C-3′ or C-2′, respectively. All $^1H$ and $^{13}C$ NMR resonances were easily attributed by analogy with austrodorin **5**.[17] Acetylation of aliquots of both compounds gave the diacetyl derivative **6**,[18] identical in all respects with diacetyl austrodorin previously characterized,[9] further confirming the proposed structure. In order to assign the absolute stereochemistry at C-2′ of the diacylglycerol **1**, the modified Mosher method[19,20] was applied. The observed $\Delta\delta$ ($\delta S$–$\delta R$) in the $^1H$ NMR chemical shifts (Table 1) of the (*S*)- and (*R*)-esters, **7** and **8**,[21] obtained by treating **1** with (*R*)- and (*S*)-α-methoxy-α-trifluoromethylphenylacetic (MTPA) chloride, respectively, suggested an *R* absolute stereochemistry at C-2′.

Two specimens of *A. kerguelenensis* (10 and 5 cm length, respectively), were caught by trawling, in the Weddell Sea, Antarctica, within the framework of a German expedition,[22] in February 1996, and subsequently transferred to the ICMIB. One frozen individual (size 10 cm) was immersed in acetone, using ultrasonic vibration, to extract only the metabolites present in the external part of the nudibranch. The animal was then extracted with acetone. The $Et_2O$ fractions of both acetone extracts were compared by TLC, revealing the presence of some metabolites at $R_f$ 0.35 (petr. ether:$Et_2O$, 1:1), exclusively in the extract of the external part (43 mg), which was submitted to a silica gel column chromatography

Table 1
Selected $\delta^1$H NMR[a,b] values for Mosher's esters of compounds **1** and **2**

| Position | $\delta^1$H (*S*)-MTPA ester **7** | $\delta^1$H (*R*)-MTPA ester **8** | Δδ (δS-δR) | $\delta^1$H (S)-MTPA ester **9** | $\delta^1$H (R)-MTPA ester **10** | Δδ (δS-δR) |
|---|---|---|---|---|---|---|
| 14 | 1.99 | 2.06 | - 0.07 | 2.00 | 2.10 | - 0.10 |
| | 2.23 | 2.30 | - 0.07 | 2.28 | 2.35 | - 0.07 |
| 16 | 0.86 | 0.88 | - 0.02 | 0.93 | 0.95 | - 0.02 |
| 1' | 4.33 | 4.43 | - 0.10 | 4.33 | 4.43 | - 0.10 |
| | 4.11 | 4.17 | - 0.06 | 4.13 | 4.19 | - 0.06 |
| 3' | 4.42 | 4.33 | + 0.09 | 4.42 | 4.33 | + 0.09 |
| | 4.18 | 4.12 | + 0.06 | 4.18 | 4.13 | + 0.05 |
| Ac | 2.06 | 1.94 | + 0.12 | 2.06 | 1.99 | + 0.07 |

[a] 500 MHz, $CDCl_3$, chemical shifts are referenced to $CHCl_3$ (δ 7.26). [b]Assignments were aided by $^1$H-$^1$H COSY and HMBC experiments.

using a petroleum ether/$Et_2O$ gradient as eluent. The fraction eluted by petr. ether:$Et_2O$, 1:1 (8.5 mg) was purified by normal-phase HPLC (*n*-hexane:isopropanol, 99:1) to give the known 1,3-diacylglycerol **2** (2.1 mg) and 1,2-diacylglycerol **4** (5.2 mg), which were identified by $^1$H NMR and $[\alpha]_D$,[15,23] whereas, the other metabolites described in the previous paper[15] were absent. As the absolute stereochemistry at C-2′ of the glyceryl moiety had not been previously assigned,[15] the modified Mosher method was applied to compound **2**. Analogously with **1**, compound **2** was treated with (*R*)- and (*S*)-MTPA chloride, to give the (*S*)- and (*R*)-esters, **9** and **10**,[24] respectively. The observed Δδ (δ*S*–δ*R*) in the $^1$H NMR chemical shifts (Table 1) of the esters **9** and **10** indicated the *R* absolute configuration at C-2′, in keeping with **1**.

Surprisingly, both diacylglycerols **1** and **2**, from two distinct populations of *A. kerguelenensis*, exhibited the 2′-(*R*) configuration, which was opposite to that so far reported for all terpenoid diacylglycerols from marine dorid nudibranchs collected at different geographical areas, such as the Mediterranean Sea as well as Atlantic or Pacific Oceans.[6–8,10–14,25,26] This finding could be related to the peculiarities of an Antarctic marine ecosystem, which is, among others, characterized by low temperatures and a pronounced seasonality. This undoubtedly influences the metabolism of living organisms. Further studies will be required to investigate biological activities of 2′-(*R*)-diacylglycerols in comparison with those displayed by 2′-(*S*)-diacylglycerols.[5]

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14. Ungur, N.; Gavagnin, M.; Fontana, A.; Cimino, G. *Tetrahedron: Asymmetry* **1999**, *10*, 1263–1273.
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16. BENTART 95 (AGASSIZ); depth of collection 49–233 m.
17. Compound **1**: $[\alpha]_D$ 49.4 (c 0.26, $CHCl_3$); IR: $\nu_{max}$ (liquid film) 1742 $cm^{-1}$; $^1H$ NMR: $\delta_H$ (400 MHz, $CDCl_3$) 5.30 (1H, m, H-1), 4.21–4.12 (4H, m, $H_2$-1′and $H_2$-3′), 4.10 (1H, m, H-2′), 2.35 (1H, dd, *J*=14.9 and 5.7 Hz, H-14a), 2.10 (1H, dd, *J*=14.9 and 8.6 Hz, H-14b), 2.11 (3H, s, OAc), 0.97 (3H, s, $H_3$-20), 0.91 (3H, d, *J*=6.6 Hz, $H_3$-16), 0.84 (6H, s, $H_3$-18 and $H_3$-19), 0.83 (3H, d, overlapped, $H_3$-17); $^{13}C$ NMR: $\delta_C$ (100 MHz, $CDCl_3$) 173.4 (C-15), 171.0 (OAc), 146.2 (C-10), 116.8 (C-1), 68.4 (C-2′), 65.3 (C-1′ or C-3′), 65.0 (C-3′ or C-1′), 44.6 (C-8), 43.6 (C-5), 42.5 (C-9), 41.8 (C-14), 31.4 (C-7), 31.3 (C-4 and C-13), 31.2 (C-3), 30.6 (C-12), 30.2 (C-6), 28.2 (C-11), 28.0 (C-18 or C-19), 27.4 (C-19 or C-18), 23.2 (C-2 and C-20), 20.8 (OAc), 20.0 (C-16), 16.4 (C-17); EIMS *m/z* (%) 422 ($M^+$, 7), 407 (5), 349 (7), 289 (25), 245 (3), 192 (100), 135 (94). Compound **3**: $[\alpha]_D$ 51.2 (c 1.1, $CHCl_3$); IR: $\nu_{max}$ (liquid film) 1740 $cm^{-1}$; $^1H$ NMR: $\delta_H$ (400 MHz, $CDCl_3$) 5.29 (1H, m, H-1), 5.10 (1H, quintet, *J*=5.3 Hz, H-2′), 4.31 (1H, dd, *J*=12.0 and 4.4 Hz, $H_2$-1′a), 4.23 (1H, dd, *J*=12.0 and 5.9 Hz, $H_2$-1′b), 3.73 (2H, m, $H_2$-3′), 2.35 (1H, dd, *J*=14.7 and 5.6 Hz, H-14a), 2.11 (1H, dd, *J*=14.7 and 8.7 Hz, H-14b), 2.08 (3H, s, OAc), 0.97 (3H, s, $H_3$-20), 0.92 (3H, d, *J*=6.7 Hz, $H_3$-16), 0.84 (6H, s, $H_3$-18 and $H_3$-19), 0.83 (3H, d, overlapped, $H_3$-17); $^{13}C$ NMR: $\delta_C$ (100 MHz, $CDCl_3$) 173.0 (C-15), 171.0 (OAc), 146.2 (C-10), 116.8 (C-1), 72.0 (C-2′), 62.3 (C-1′ or C-3′), 61.6 (C-3′ or C-1′), 44.6 (C-8), 43.6 (C-5), 42.5 (C-9), 41.9 (C-14), 31.3 (C-4, C-7 and C-13), 31.2 (C-3), 30.6 (C-12), 30.2 (C-6), 28.2 (C-11), 28.0 (C-18 or C-19), 27.6 (C-19 or C-18), 23.2 (C-2 and C-20), 20.7 (OAc), 19.8 (C-16), 16.4 (C-17); EIMS *m/z* (%) 422 ($M^+$, 3), 407 (2), 349 (3), 289 (16), 192 (80), 191 (100).
18. Compound **3** (2.1 mg) was dissolved in dry pyridine (1 ml) and 1 drop of acetic anhydride was added. The mixture was stirred at rt for 12 h. After removing the solvent, the crude reaction product was purified by $SiO_2$ Pasteur pipette (petr. ether/$Et_2O$ gradient), to give 2.4 mg of pure **6**, which was identical to diacetyl-austrodorin (Ref. 9). Compound **1** (0.5 mg) was acetylated by using the above procedure to give 0.5 mg of **6**.
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21. (*S*)- and (*R*)-MTPA esters of compound **1** were prepared by treating two aliquots of 0.6 mg each of **1** respectively with (*R*)- and (*S*)-MTPA chloride (an excess) in dry pyridine (0.5 ml), for 12 h at rt under stirring. The esters were purified by chromatography on silica gel contained in a Pasteur pipette (petr. ether/$Et_2O$ gradient), obtaining 0.3 mg of **7** and 0.4 mg of **8**. $^1H$ NMR resonances of esters **7** and **8** (significant selected data are reported in Table 1) were assigned by $^1H$–$^1H$ COSY and HMBC experiments.
22. ANT XIII/3 (EASIZ I); depth of collection 246–468 m.
23. Compound **2**: 2.1 mg; $[\alpha]_D$ −51 (c 0.2, $CHCl_3$), lit.[15] $[\alpha]_D$ −53 ($CHCl_3$); $^1H$ NMR: $\delta_H$ (400 MHz, $CDCl_3$) 4.21–4.11 (4H, m, $H_2$-1′ and $H_2$-3′), 4.10 (1H, m, H-2′), 2.40 (1H, dd, *J*=15.0 and 5.8 Hz, H-14a), 2.17 (1H, dd, *J*=15.0 and 8.3 Hz, H-14b), 2.11 (3H, s, OAc), 1.54 (3H, bs, $H_3$-17), 0.98 (3H, d, *J*=6.7 Hz, $H_3$-16), 0.92, 0.87 and 0.82 (3H each, 3 s, $H_3$-18, $H_3$-19 and $H_3$-20). Compound **4**: 5.2 mg; $[\alpha]_D$ −52 (c 0.5, $CHCl_3$), lit.[15] $[\alpha]_D$ −48 ($CHCl_3$); $^1H$ NMR: $\delta_H$ (400 MHz, $CDCl_3$) 5.10 (1H, quintet, *J*=5.3 Hz, H-2′), 4.31 (1H, dd, *J*=12.0 and 4.4 Hz, $H_2$-1′a), 4.23 (1H, dd, *J*=12.0 and 5.9 Hz, $H_2$-1′b), 3.74 (2H, m, $H_2$-3′), 2.41 (1H, dd, *J*=14.8 and 5.7 Hz, H-14a), 2.17 (1H, dd, *J*=14.8 and 8.4 Hz, H-14b), 2.08 (3H, s, OAc), 1.54 (3H, bs, $H_3$-17), 0.98 (3H, d, *J*=6.6 Hz, $H_3$-16), 0.92, 0.87 and 0.82 (3H each, 3 s, $H_3$-18, $H_3$-19 and $H_3$-20).
24. Esters **9** (0.4 mg) and **10** (0.4 mg) were prepared by using the procedure reported in Ref. 21. Selected $^1H$ NMR resonances are reported in Table 1.
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26. Mosher's method was also applied to these diacylglycerols confirming the 2′-(*S*)-configuration previously established either by chemical methods (Ref. 25) or by synthesis (Refs. 10, 11 and 13).