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Austrodorin-A and -B: first tricyclic diterpenoid 2′-monoglyceryl esters from an Antarctic nudibranch

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Abstract

Two novel diterpenoid 2′-monoglyceryl esters, austrodorin-A (**7**) and austrodorin-B (**8**), which contain the isocopalane skeleton with an uncommon oxygenated substitution pattern, have been isolated from the skin of the Antarctic nudibranch *Austrodoris kerguelenensis*. Structures and relative stereochemistries have been established by NMR methods, whereas the absolute configuration was suggested by comparison of their CD profiles with those of model compounds.

Keywords: marine metabolites; molluscs; terpene; terpenoids; glycerol.

Austrodoris kerguelenensis, Bergh 1884, is a relatively common nudibranch, widely distributed in the High Antarctic and Subantarctic zone. Analogously with other dorid nudibranchs belonging to related genera,[1–10] this mollusc is characterized by the presence of terpenoid glyceryl esters, most likely de novo biosynthesized. These molecules, which are supposed to be involved in the defensive mechanisms of the animal, display very interesting biological acivities, including the activation of protein kinase C.[11] Previous studies on different collections of *A. kerguelenensis* have led to a series of *ent*-labdane diterpenoid glycerols (e.g. **1**)[12] and of the halimane diterpenoid glycerol, austrodorin (**2**).[13] Recently, we have reported the assignment of the *R* absolute configuration at C-2′ of the glyceryl moiety of the 1,3-glyceryl esters **3** and **4**,[14] isolated along with the corresponding 1,2-derivatives, **5** and **6**, from the skin of two distinct populations of *A. kerguelenensis*.[12,14] The ethereal extract from the specimens of one of these collections also contained a mixture of unrelated terpenoid monoacylglycerols, which were not further investigated.

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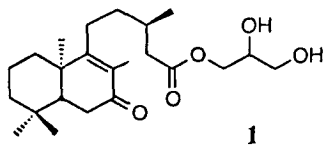

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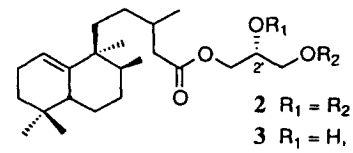
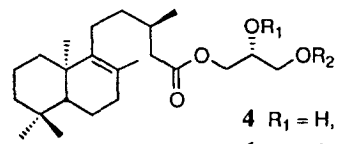
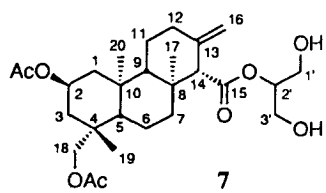


2 $R_1 = R_2 = H$
3 $R_1 = H$, $R_2 = Ac$
5 $R_1 = Ac$, $R_2 = H$



4 $R_1 = H$, $R_2 = Ac$
6 $R_1 = Ac$, $R_2 = H$

We describe here the structural elucidation of the two components of this mixture, austrodorin-A (**7**) and austrodorin-B (**8**), which are 2′-monoglyceryl esters of new oxygenated isocopalane acids. As previously reported,[14] the mantle, the digestive gland and the mucus of two specimens of *A. kerguelenensis*, collected at South Shetland Islands (Antarctica) in 1995, were separately extracted with acetone. A series of compounds at R_f 0.5–0.1 (petroleum ether:Et_2O, 3:7) were present only in the mantle and in the mucus extracts. The combined extracts (330 mg) were fractionated by silica gel chromatography (petroleum ether/Et_2O gradient) giving **3** (4.4 mg) and **5** (22.4 mg), as already described,[14] and a more polar fraction (100 mg), which was further purified (silica gel column, $CHCl_3$:MeOH gradient; reverse-phase HPLC, MeOH:H_2O, 7:3), to give austrodorin-A (**7**, 10.3 mg) and austrodorin-B (**8**, 3.5 mg).



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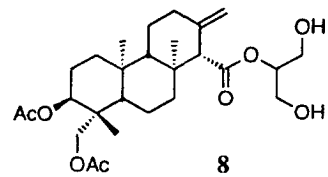

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Austrodorin-A (**7**)‡ exhibited the molecular formula $C_{27}H_{42}O_8$, deduced from HREIMS on *m/z* 494. The ^1H and ^{13}C NMR spectra (Table 1) revealed the presence of a glyceryl residue esterified at C-2′ with a diterpenoid acid and of two acetyl groups. In addition, three methyl singlets at δ 0.96, 1.02 and 1.06, two methine signals at δ 4.69 (d, J=0.8 Hz) and 4.85 (br s), an AB system at δ 3.95 (1H, d, J=11.2 Hz) and 4.10 (1H, d, J=11.2 Hz), suggested a tricyclic isocopalane skeleton exhibiting an exomethylene group and an oxidized tertiary methyl. The proton spectrum also showed a broad multiplet at δ 4.99 (1H, $w_{1/2}$=20 Hz), which was assigned to an axial methine (H-2), further coupled with two methylenes, each linked to a quaternary carbon. These data supported the location of the secondary acetyl function in the ring A at C-2, whereas the placement of the acetoxymethyl group at C-4 was indicated by diagnostic correlations in the HMBC spectra of **7** (Table 1). In particular, H_2-18 displayed long-range connectivities with C-3, C-4, C-5 and C-19. The equatorial orientation of H_3-19 was suggested by the ^{13}C NMR value of C-19 (δ 27.64). NOE interactions among H-2, H_2-18 and H_3-20, and between H-14 and H-9 confirmed the suggested relative stereochemistry. Extensive 2D NMR studies (^1H–^1H COSY, ^1H–^1H TOCSY, HMQC, HMBC), along with homonuclear decoupling experiments, allowed complete NMR assignments (Table 1), which were in good agreement with those reported in the literature for terpenoid models containing either the

‡ $[\alpha]_D$ 22.9 (*c* 0.18, $CHCl_3$); $[\theta]_{223}$ (EtOH) 1580; IR (liquid film): 3440, 1734 cm^{-1}; HREIMS found: 494.2900 (M^+); $C_{27}H_{42}O_8$ requires: 494.2880; EIMS (%): 494 (3), 434 (2), 402 (40), 387 (8), 361 (13), 342 (14), 269 (50), 119 (100).

Table 1. NMR data[a,b] for austrodorin-A (**7**) and austrodorin-B (**8**)

Position	Austrodorin-A (**7**)			Austrodorin-B (**8**)		
	δ ^{1}H m, *J*, Hz	δ ^{13}C m[c]	Long range connectivities[d]	δ ^{1}H m, *J*, Hz	δ ^{13}C m[c]	Long range connectivities[d]
1	0.88 m	45.07 t	H-2, H_2-3, H_3-20	1.22 m	33.55 t	H-3, H_3-20
	2.10 m			1.50 m		
2	4.99 m, $w_{1/2}$ = 20Hz	67.96 d	H_2-1, H_2-3	1.68 m	22.19 t	H-1a
				1.85 m		
3	1.05 m	41.18 t	H-2, H_2-18, H_3-19	4.94 m, $w_{1/2}$ = 7Hz	73.38 d	H_2-18, H_3-19
	2.05 m					
4		38.64 s	H_2-3, H_2-18, H_3-19		40.60 s	H-5, H_2-18, H_3-19
5	1.07 m	56.66 d	H_2-1, H_2-6, H_2-18, H_3-19, H_3-20	1.50 m	50.85 d	H-3, H-7a, H_3-19, H_3-20
6	1.45 m	18.60 t	H-5, H_2-7	1.40 m	18.36 t	H-5
	1.75 m			1.60 m		
7	1.30 m	40.38 t	H-9	1.35 m	40.31 t	H_3-17
	1.70 m			1.70 m		
8		39.61 s	H_2-7, H-14		39.67 s	H-14, H_3-17
9	1.11 m	59.15 d	H-1a, H-7a, H-12a, H-14, H_3-17, H_3-20	1.16 dd, 12.5, 2.7	58.87 d	H-7a, H-14, H_3-17, H_3-20
10		39.11 s	H-1a, H_3-20		37.25 s	H-5, H_3-20
11	1.45 m	22.37 t	H-9, H_2-12	1.45 m	22.47 t	
	1.65 m			1.65 m		
12	2.02 m	35.82 t	H-9, H-11a, H-14, H_2-16	2.10 m	35.89 t	H-14, H_2-16
	2.43 m			2.44 m		
13		143.00 s	H-12a, H-14, H-16a		143.19 s	H-12a, H-14, H-16a
14	2.85 s	63.05 d	H-12a, H_2-16, H_3-17	2.91 s	63.05 d	H-12b, H_2-16, H_3-17
15		171.29 s	H-14, H-2'		171.35 s	H-14, H-2'
16	4.69 d, 0.8	108.69 t	H_2-12, H-14	4.70 d, 1.0	108.52 t	H-14
	4.85 br s			4.85 br s		
17	1.06 s	14.94 q	H_2-7, H-9, H-14	1.07 s	14.90 q	H-14
18	3.95 d, 11.2	66.98 t	H-3a, H_3-19	3.95 d, 11.4	66.52 t	H-5, H_3-19
	4.10 d, 11.2			4.18 d, 11.4		
19	1.02 s	27.64 q	H-3a, H_2-18	0.96 s	22.19 q	H_2-18
20	0.96 s	17.54 q	H_2-1, H-5, H-9	0.88 s	16.43 q	H-5
1'	3.83 m	62.63[e] t	H-2'	3.83 m	62.66[f] t	H-2'
2'	4.96 app. quintet, 4.6	74.68 d	H_2-1', H_2-3'	4.97 app. quintet, 4.7	74.61 d	H_2-1', H_2-3'
3'	3.83 m	62.50[e] t	H-2'	3.83 m	62.54[f] t	H-2'
2- or 3-OAc	2.01 s	21.39 q		2.09 s	21.26 q	
		170.36 s	H-2, OAc		170.45 s	OAc
18-OAc	2.04 s	20.91 q		2.06 s	20.93 q	
		171.10 s	H_2-18, OAc		171.19 s	H_2-18, OAc

[a] Bruker AM 500 MHz and WM 400 MHz spectrometers, $CDCl_3$, chemical shifts (ppm) referred to $C\underline{H}Cl_3$ (δ 7.26) and to $\underline{C}DCl_3$ (δ 77.00).

[b] Assignments made by ^{1}H-^{1}H COSY, HMQC and ^{1}H-^{1}H homodecoupling experiments.

[c] By DEPT sequence.

[d] HMBC experiments (*J* = 10 Hz and 6 Hz).

[e,f] May be reversed.

same functionalized ring A[15] or the same rings B and C.[10] The absolute configuration of **7** was suggested by comparing the CD profile with those of a series of isocopalane and *ent*-isocopalane diterpenoids, the absolute stereochemistries of which have been secured by synthesis.[5,9,10,16,17]

Austrodorin-B (**8**),§ with the molecular formula $C_{27}H_{42}O_8$ deduced from both EIMS and ^{13}C NMR data, was an isomer of **7**. The NMR patterns (Table 1) were essentially the same, but the chemical shifts differed, with the largest changes occurring for resonances of ring A. This suggested a structure only differing from **7** in the position in the ring A of the secondary acetoxy group. A sharp multiplet at δ 4.94 (1H, $w_{1/2}$=7 Hz, H-3), connected to a methylene (H_2-2, δ 1.68 and 1.85), in turn coupled with another methylene at δ 1.22 and 1.50 (H_2-1), linked to a quaternary carbon (C-10), located the secondary acetyl group of **8** axially at C-3. The ^{13}C NMR spectrum showed signals for C-1, C-2 and C-3 consistent with the proposed substitution pattern and in agreement with the literature.[18] The axial orientation of the acetoxymethyl group at C-4 was suggested by ^1H and ^{13}C chemical shift values, almost identical to those of **7**, whereas a significantly different resonance was observed for C-19 (δ 22.19), upfield-shifted by the substituent at C-3. The relative configuration of the chiral centres was supported by a series of NOE experiments, which connected H_3-20 to H-18a and H_3-17, confirming their *cis*-relationship. Steric interactions were observed also between H-9 and H-14; H-5 and H_3-19; H-3 and H_3-19, according to the proposed structure **8**. All NMR resonances were assigned by 2D experiments (Table 1). As for **7**, the isocopalane absolute configuration of **8** was suggested by comparing the diagnostic CD curve with those of model compounds.

Several terpenoid acylglycerols have been isolated from dorids in the last few years.[1–10,12–14] The majority of these molecules have the terpenoid residue linked to the 1-*sn* position of glycerol, which is further esterified by an acetyl function at the 2-*sn* or 3-*sn* position. Biosynthesis de novo of diacylglycerols in dorid nudibranchs has also been recently demonstrated.[19] The monoglyceryl esters austrodorin-A and -B display two diterpene isocopalane residues, which are characterized by an oxygenation pattern uncommon in marine organisms, linked to C-2 of glycerol. This unusual position of esterification in terpenoid glyceryl esters has been, until now, reported only for the Atlantic *Archidoris tuberculata*[6] and the Pacific *Sclerodoris tanya*.[8] However, this is the first finding of tricyclic diterpenoids in an Antarctic nudibranch. It has been reported that de novo biosynthesis should lead to the same metabolites in the same mollusc.[20] Surprisingly, distinct collections of *A. kerguelenensis* seem to be able to construct different mantle metabolite patterns.

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