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Austrodoral and austrodoric acid: *nor*-sesquiterpenes with a new carbon skeleton from the Antarctic nudibranch *Austrodoris kerguelenensis*

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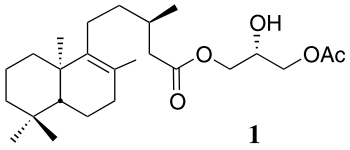
Abstract—Two unprecedented *nor*-sesquiterpenes, austrodoral (**4**) and its oxidised derivative austrodoric acid (**5**), have been isolated from the skin of the marine dorid *Austrodoris kerguelenensis*, collected in Antarctica. The structures and the relative stereochemistry were elucidated on the basis of spectroscopic data. A role of stress-metabolites could be suggested for these compounds. © 2003 Elsevier Science Ltd. All rights reserved.

Austrodoris kerguelenensis, Bergh 1884, is a common Antarctic nudibranch, widely distributed in the High Antarctic and Subantarctic Zone. Analogously with other dorid nudibranchs belonging to related genera,[1–10] this mollusc is characterised by the presence in its skin of a series of terpenoid glyceryl esters, which are involved in the defensive mechanisms of the animal.[11] Previous studies on different collections of *A. kerguelenensis* have led to a series of *ent*-labdane (e.g. **1**),[12,13] halimane (e.g. austrodorin, **2**),[13,14] and isocopalane diterpenoid glycerols (e.g. austrodorin-B, **3**).[15]



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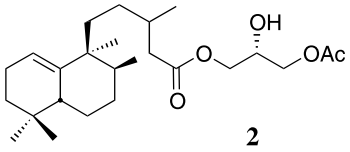

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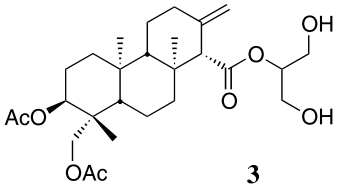

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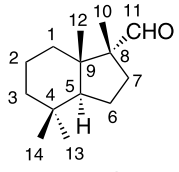

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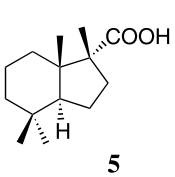

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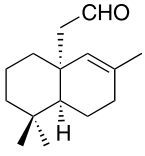

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Keywords: marine natural product; terpenes and terpenoids; nudibranch.

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In the course of our study on the chemical ecology of marine nudibranchs we have chemically investigated a population of *A. kerguelenensis* collected off Terra Nova Bay, Antarctica. In addition to the expected terpenoid glyceryl ester fraction, which has been analysed only preliminarily, two metabolites exhibiting an unprecedented *nor*-sesquiterpene carbon skeleton have been found in the ether extract of the skin of the mollusc.

We describe here the structural elucidation of these two novel *nor*-sesquiterpenes, austrodoral **4** and austrodoric acid **5**, the structures of which are unrelated to those of all terpenoid acylglycerols derivatives isolated so far from *A. kerguelenensis*.

Twelve individuals of *A. kerguelenensis* were collected by SCUBA at depths of 10–20 meters, off Terra Nova Bay, Antarctica, during the Austral Summer 1999–2000, in the frame of the 15th Italian Expedition. Seven specimens were put immediately at −20°C, whereas the remaining five animals were kept in an aquarium for 15 days, before being frozen. All biological material was then transferred to Italy and chemically analysed. Each specimen was carefully dissected into mantle and internal organs, that were separately extracted by acetone. A comparative chromatographic analysis of the diethyl ether soluble part from the acetone extract of both mantle and internal organs for each individual revealed different metabolite patterns for the two parts, in agreement with already reported results.[12–15] A series of terpenoid compounds at R_f 0.70 and 0.25 (light petroleum/diethyl ether, 8:2), and at R_f 0.75 and 0.35 (light petroleum/diethyl ether, 2:8) giving a pink coloration by spraying with $CeSO_4$ were detected only in the mantle extract of the animals. However, a different relative distribution of these metabolites was observed for each specimen. In particular the two less polar compounds were found to be present in traces in those individuals which were frozen immediately after the capture, while they were detected at significantly higher levels (10–20 times) in the five animals kept in captivity for two weeks before being frozen.

All mantle ether extracts were combined (605 mg) and chromatographed by Si-gel column (light petroleum ether/diethyl ether gradient) to give four fractions containing terpenoid metabolites. Preliminary ^{1}H NMR analysis revealed that the two more polar fractions were constituted by mixtures of diterpene glyceryl esters, as already reported, whereas the less polar fractions contained terpenes not related to the previously known compounds.[12–15] These two fractions were further purified by HPLC (normal phase, Spherisorb 5μ, *n*-hexane/ethyl acetate, 9:1) to give two new molecules that we named austrodoral (**4**, 7.9 mg)[†] and austrodoric acid (**5**, 2.5 mg).[‡] The close relationship between **4** and **5** was immediately revealed by comparison of their spectral data and further confirmed by observing the partial conversion of **4** into **5** during work-up. Both compounds were unstable.

The molecular formula $C_{14}H_{24}O$ of austrodoral (**4**) was deduced by both EIMS and ^{13}C NMR spectra. Mass spectrum showed a peak at m/z 209, corresponding to M+1 ion, while in the ^{13}C NMR spectrum 13 signals, attributable to sp^3 carbons (four CH_3, five CH_2, one CH and three C as deduced by DEPT sequence), and a signal a δ 207.9, due to an aldehyde carbonyl linked to a saturated carbon, were observed. The remaining two unsaturation degrees indicated by molecular formula were therefore attributed to two rings. ^{1}H NMR spectrum displayed a 1H singlet at δ 9.68 (H-11), assigned to the aldehyde proton, four 3H singlets at δ 0.86 (H_3-13), 0.88 (H_3-12), 0.89 (H_3-14) and 1.04 (H_3-10) attributed to four tertiary methyls, and multiplets integrating for eleven protons between δ 2.16 and 0.97 assigned to five methylene and one methine groups by ^{13}C–^{1}H heterocorrelation (HSQC). Analysis of a ^{1}H–^{1}H COSY experiment led to establish the methylene sequence. In particular the protons resonating at δ 1.65 and 1.20 (H_2-1) were coupled with H_2-2 (δ 1.60 and 1.51), which was further correlated with protons at δ 1.42 and 0.97 (H_2-3), whereas the methylene resonating at δ 2.16 and 1.30 (H_2-7), linked to the quaternary carbon bearing a -CHO group, resulted to be coupled with protons at δ 1.68 and 1.41 (H_2-6), further correlated to the methine proton at δ 1.25 (H-5). These data suggested a *nor*-sesquiterpene bicyclic skeleton as shown in **4**, probably derived from the drimane framework by contraction of ring B and loss of a carbon atom. HMBC spectrum of compound **4** showed significant correlations between C-9 (δ 58.3) and protons at δ 0.88 (H_3-12), 1.04 (H_3-10) and 2.16 (H-7a) and between C-7 (δ 28.6) and protons at δ 1.04 (H_3-10) and 9.68 (H-11), confirming the suggested structure. All NMR values were assigned (Table 1). Unfortunately, compound **4** fully degraded in NMR tube preventing the recording of NOE experiments. However, comparison of ^{13}C NMR values of methyls H_3-12, H_3-13 and H_3-14 and of carbons in ring A with the literature data for the corresponding carbons of drimane skeleton strongly supported a *trans*-junction between rings A and B, whereas the relative stereochemistry at C-8 with the α-orientated aldehydic group was suggested by analysis of NMR data of related compound **5**.

The molecular formula $C_{14}H_{24}O_2$ of austrodoric acid (**5**) was deduced by HREIMS on molecular peak at m/z 224. ^{1}H and ^{13}C NMR spectra of **5** were substantially similar with those of **4** (Table 1), clearly indicating that the unique difference between the two metabolites was in the oxidation degree at C-11. In particular, ^{13}C NMR spectrum of **5** displayed a signal at δ 179.8

[†] $[\alpha]_D$ 5 (*c* 0.3, $CHCl_3$); EIMS (%) 209 (M+1, 10), 179 (2), 163 (8), 138 (15), 123 (100), 109 (20), 95 (24), 82 (15).

[‡] $[\alpha]_D$ 16 (*c* 0.1, $CHCl_3$); IR (liquid film) 1687 cm^{-1}; EIMS (%) 224 (25), 209 (100), 163 (17), 138 (10), 123 (40), 82 (10); HRESIMS (negative mode) found 223.1696 $(M-H)^-$, $C_{14}H_{23}O_2$ requires 223.1698.

Table 1. NMR data[a,b] for austrodoral (**4**) and austrodoric acid (**5**)

	Austrodoral (**4**)			Austrodoric acid (**5**)		
Position	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	1.65 m 1.20 m	33.1 t	H_2-3, H-5, H_3-12	1.58 m 1.12 ddd, 13,12,6	35.3 t	H-3a, H_3-12
2	1.60 m 1.51	19.6 t	H-1a, H-3a	1.60 m 1.52 m	20.1 t	H-1a, H-3b
3	1.42 m 0.97 ddd, 13,13,4	41.2 t	H-1b, H_3-13, H_3-14	1.42 m 1.02 ddd, 13,13,4	41.2 t	H-1b, H-5, H_3-13, H_3-14
4	–	33.2 s	H-6a, H_3-13, H_3-14	–	33.2 s	H-6a, H_3-13, H_3-14
5	1.25 m	54.7 d	H_2-6, H_3-12, H_3-13, H_3-14	1.62 m	53.3 d	H_2-6, H_3-12, H_3-13, H_3-14
6	1.68 m 1.41 m	21.4 t	H-5, H-7a	1.70 m 1.45 m	21.6 t	H-5, H_3-12, H_3-13
7	2.16 ddd, 13,10,6 1.30 m	28.6 t	H-5, H_2-6, H_3-10, H-11	2.31 m 1.43 m	33.1 t	H-6a, H_3-10
8	–	46.6 s	H_2-1, H-5, H-6a, H_3-10, H_3-12	–	46.7 s	H-5, H-6b, H-7a, H_3-10, H_3-12
9	–	58.3 s	H-7a, H_3-10, H_3-12	–	56.4 s	H-6b, H_3-10, H_3-12
10	1.04 s	16.4 q	H-7b	1.15 s	20.5 q	H-7b
11	9.68 s	207.9 d	H_2-7, H_3-10	–	179.8 s	H_2-7, H_3-10
12	0.88 s	15.9 q	H_2-1, H-5	0.87 s	15.7 q	H_2-1, H-5
13	0.86 s	33.6 q	H-3a, H_3-14	0.87 s	33.8 q	H-5, H_3-14
14	0.89 s	21.4 q	H-5, H_3-13	0.90 s	21.6 q	H-5, H_3-13

[a] Bruker DPX 500 and AVANCE 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 77.0).
[b] Assignments made by ^{1}H–^{1}H COSY, HSQC and ^{1}H–^{1}H homodecoupling experiments.
[c] By DEPT sequence.
[d] HMBC experiments ($J = 10$ and 6 Hz).

replacing the signal at δ 207.9 (C-11) and in addition significant differences were observed for the resonances of C-7 and C-10 (Table 1). In the ^{1}H NMR spectrum, the signal at δ 9.68 was obviously missing and, analogously with the carbon values, the resonances of protons H_3-10 (δ 1.15) and H_2-7 (δ 1.43 and 2.31) differed from those of austrodoral (**4**). But the most significant shift was observed for H-5 resonating at δ 1.62 (δ 1.25 in **4**), clearly indicating a strong influence on H-5 by the presence of oxidised substituent at C-8 and then supporting the relative stereochemistry at this centre with the α-orientated -COOH group. A NOESY correlation between H_3-12 and H_3-14 further confirmed the proposed relative configuration at C-8.

The degradation of the *nor*-sesquiterpenes pair **4** and **5** prevented the evaluation of their biological activity. However, by considering that the conversion of the aldehyde **4** into the acid **5** has been observed to occur in solution, it seems probable that the aldehyde **4** is the natural metabolite, whereas the oxidised derivative **5** is a work-up product. The presence of austrodoral (**4**) in the external part of the animal could be related to some defensive mechanism, even though it has been demonstrated that a defensive role in *A. kerguelenensis* is played by the co-occurring terpenoid acylglycerols.[11] On the other side, it is interesting to note that the level of compound **4** (and of its derivative **5**) detected in the animal was significantly more abundant in those individuals kept in aquarium for 15 days before freezing, suggesting for austrodoral (**4**) a probable role of stress-metabolite.

Sesquiterpene aldehydes exhibiting cyclic rearranged skeletons (e.g. isoacanthodoral, **6**) have been reported from the skin of British Columbia dorid nudibranch, *Acanthodoris nanaimoensis*.[16,17] A recent investigation on the biosynthesis of these compounds by using ^{13}C-labelled acetate showed that they are formed de novo by the mollusc and a plausible pathway from a monocyclofarnesane precursor was also suggested.[18]

Austrodoral (**4**), which was absent in the digestive gland of the mollusc, could be derived in *A. kerguelenensis* by a biosynthetic scheme similar to that described for *A. nanaimoensis* sesquiterpenes.

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