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New cytotoxic isomalabaricane-type sesterterpenes from the New Caledonian marine sponge *Rhabdastrella globostellata*

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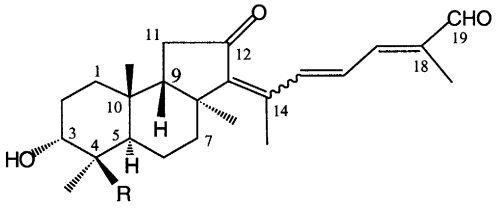
Abstract

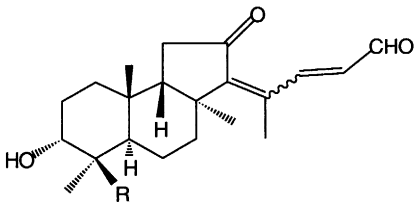
Four new cytotoxic isomalabaricane sesterterpenes, aurorals **1**–**4**, have been isolated from the New Caledonian marine sponge *Rhabdastrella globostellata*. Their structures were established by spectroscopic data including 1D and 2D techniques. These compounds were found to exhibit cytotoxic activity on KB cells. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: marine sponge; *Rhabdastrella globostellata*; aurorals; isomalabaricane terpenoids; cytotoxic compounds.

Marine sponges continue to be a fruitful source of unusual terpenoids. Recently, we examined the cytotoxic dichloromethane extract of the marine sponge *Rhabdastrella globostellata* (previously designated *Aurora sp.*) collected off New Caledonia, and isolated four new cytotoxic sesterterpenes, **1**–**4**, named aurorals. These compounds represent the first natural isomalabaricane-type terpenoids with a primary alcohol at C-4 position. A complete chemical dataset of these new compounds and results of the cytotoxic assays on KB cells are presented here.



1: R = CH_2OH, Δ^{13}=*Z*
2: R = CH_2OH, Δ^{13}=*E*
5: R = COOH, Δ^{13}=*Z*
6: R = COOH, Δ^{13}=*E*



3: R = CH_2OH, Δ^{13}=*Z*
4: R = CH_2OH, Δ^{13}=*E*
7: R = COOH, Δ^{13}=*Z*
8: R = COOH, Δ^{13}=*E*

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Lyophilized specimens (700 g) were extracted at room temperature in dichloromethane for two days. The resulting bright-yellow colored crude extract (12 g) exhibited promising cytotoxic activity on KB cells. Bioassay-guided fractionation by silica gel chromatography using a linear gradient of MeOH in CH_2Cl_2 as eluent, furnished two active fractions. The first one, eluted with CH_2Cl_2:MeOH 95:5 (3 g) was successively fractionated by aluminium oxide basic flash chromatography, and preparative C_{18} TLC (MeOH:H_2O 8:2) to afford a 1:1 mixture of compounds **1** and **2** (0.00028% dry weight). The second one, eluted with CH_2Cl_2:MeOH 9:1 (4 g) was chromatographed on a C_{18} column (MeOH:H_2O 8:2), then on a silica gel column (hexane:AcOEt 5:5) to yield a 1:1 mixture of compounds **3**–**4** (0.00055% dry weight). The presence of a mixture of two isomers was in each case suggested by the ^{1}H and ^{13}C NMR spectra, which exhibited some resonances as double peaks. EI spectra, which showed no peak greater than *m/z* 400 and 360 for compounds **1**–**2** and **3**–**4**, respectively, confirmed this proposal. Attempts to separate the isomers by normal or reversed-phase columns were unsuccessful. We decided to continue structural elucidation on the basis of the 1H and 2D NMR data.

The molecular formula of compounds **1** and **2** were established as $C_{25}H_{36}O_4$ [$(M+H)^+$, 401.2692, Δ 0 mmu] by HRMS, indicating eight unsaturations in the molecules. The ^{13}C NMR spectrum established the presence of a ketone carbonyl (δ 206.8 ppm), an aldehyde (δ 194.9/194.6 ppm), a secondary alcohol (δ 71.4 ppm) and a primary alcohol (δ 67.8 ppm). This was supported by absorption bands at ν 3447, 1740 and 1670 cm^{-1} in the IR spectrum. Further resonances in the ^{13}C NMR spectrum at δ 149.5/150.1, 139.5/139.7, 140.5/140.5, 128.1/129.5, 148.9/147.6, 139.5/140.4 ppm established the presence of three carbon–carbon double bonds which, from UV absorption at 342 nm (ε 9000) suggested that the carbon–carbon double bonds and the carbonyl must be conjugated. Hence, the presence of three double bonds, one carbonyl and one aldehyde group indicated that compounds **1** and **2** must therefore be tricyclic. Further structural informations were obtained from the ^{1}H NMR spectrum of **1** and **2**, which indicated the presence of two vinyl methyl groups with resonances at δ 2.04/2.31 and 1.87/1.91 ppm and three quaternary methyl groups at δ 1.08, 1.37/1.39 and 1.02 ppm.

Full NMR data assignments for compounds **1** and **2** were obtained by careful analysis of 2D NMR data. The protonated carbons were all assigned by HMQC experiments. The signal at δ 4.04 ppm (dd, *J*=2.7, 7.2) was placed at C-3 position thanks to HMBC cross peaks with carbons C-1, C-4, C-5 and Me-4. Its coupling constants implied that the hydroxyl group was in α orientation.[1] Its COSY cross peaks with protons at δ 1.85 and 1.70 ppm, fixed protons H-2. HMBC cross peaks of the methyl protons at δ 1.02 ppm (Me-10) with C-1, C-5, C-9, C-10, of the methyl protons at δ 1.37/1.39 ppm (Me-8) with C-7, C-8, C-9, C-13, of the methyl protons at δ 1.08 ppm (Me-4) with C-3, C-4, C-5, and of the protons at δ 2.22 ppm (H-11) with C-12 and C-13, established the structure of an oxo-isomalabaricane skeleton. This structure was reminiscent of epimeric jaspiferals **5** and **6**, previously reported from the Okinawan marine sponge *Jaspis stellifera*.[2] Moreover, HMBC correlations of the pair of aldehyde protons at δ 9.47/9.50 ppm for **1** and **2**, respectively, with carbons at δ 148.9/147.6, 139.5/140.4 and 9.7/9.9 ppm established the assignment of C-17, C-18 and Me-18. COSY correlations of H-15 with the olefinic proton at δ 6.97/7.00 ppm, located the proton H-16 and allowed to deduce the connectivities in the chain. Finally, the methyl protons at δ 2.04/2.31 ppm in the chain (Me-14) showed HMBC correlations to C-13, C-15, indicating that the chain was attached to C-13.

The low-field position of the resonance for the olefinic signal at δ 8.35 ppm (1/2H, d, *J*=14.4 Hz) indicated that it was located in the deshielding region of the carbonyl group and should be assigned to H-15 of the 13-*Z*-isomer.[1,3] Hence, the proton H-15 at δ 7.01 ppm (1/2H, d, *J*=14.4 Hz), deduced from HMBC spectrum, was assigned to the 13-*E*-isomer. Moreover, the coupling constants values $J_{15,16}$=14.4 Hz and $J_{16,17}$=15.0 Hz suggested 15*E*,17*E*-geometries in both compounds.

Furthermore, information was obtained on the relative stereochemistry by NOESY experiments.

NOESY cross-peaks for H-5/Me-8 and H-9/Me-10 established the *trans–syn–trans* junction of A–C, which was also supported by ^{13}C NMR assignment of C-5 at δ 41.9/42.0 ppm for **1** and **2**.[4] Furthermore, NOESY cross-peaks for H-3/Me-10, CH_2OH/Me-10, H5/Me-4, H-9/Me-4 indicated that H-3, Me-10, CH_2OH and H-9 have the same orientation, whereas both Me-4 and Me-8 have the opposite orientation. The relative stereochemistry is thus assigned as shown.

Aurorals **3** and **4** were obtained as a 1:1 epimeric mixture at C-13. The molecular formula $C_{22}H_{32}O_4$ indicated that **3** and **4** possess seven degrees of unsaturation. Their ^1H and ^{13}C NMR spectra were similar to that of **1** and **2**, except for the absence of the methyl groups at δ 1.87/1.91 ppm and the presence of signals for only two olefinic protons at δ 8.79/7.52 ppm and 6.35/6.45 ppm. This was also supported by ^{13}C NMR resonances at δ 152.3/153.1, 137.5/138.6, 151.3/151.2 and 132.5/133.5 ppm for **3** and **4**, respectively. COSY correlations of the aldehyde protons at δ 9.66/9.68 ppm with olefinic proton at δ 6.35/6.45 ppm (H-16), which in turn showed connectivities with olefinic proton at δ 8.79/7.52 ppm (H-15) for **3** and **4**, respectively, established the structure of 3α-hydroxy-isomalabaricane terpenoids with a diene side chain (Table 1). Furthermore, NOESY experiments indicated the same relative stereochemistry as in **1** and **2**.

Table 1
^1H and ^{13}C NMR chemical shifts of compounds **1**–**4** in $CDCl_3$

N°	Compound **1**		Compound **2**		Compound **3**		Compound **4**	
	δH (m, *J*)	δC	δH (m, *J*)	δC	δH (m, *J*)	δC	δH (m, *J*)	δC
1	1.85-1.12 (m)	28.9	1.85-1.12 (m)	28.9	1.22-1.82 (m)	28.9	1.22-1.82 (m)	28.9
2	1.85-1.70 (m)	26.3	1.85-1.70 (m)	26.3	1.98-1.81 (m)	26.2	1.98-1.81 (m))	26.2
3	4.04 (dd, 2.7, 7.2)	71.4	4.04 (dd, 2.7, 7.2)	71.4	4.01 (brs)	71.3	4.01 (brs)	71.3
4		43.2		43.2		43.2		43.2
5	2.24 *(m)	41.9*	2.17* (m)	42.0*	2.24* (m)	41.8	2.28* (m)	41.8
6	1.72-1.47 (m)	19.2*	1.72-1.47 (m)	19.4*	1.69-1.42 (m)	18.3	1.69-1.42 (m)	18.3
7	2.18 (m)	38.4	2.29 (m)	40.2	2.08 (m)	38.1	2.18 (m)	41.8
8		44.8		44.9		44.8		45.2
9	1.75 (m)	50.1	1.78 (m)	49.9	1.80 (m)	49.8	1.80 (m)	49.8
10		34.9		34.9		35.1		35.1
11	2.22 (m)	36.6*	2.22 (m)	36.7*	2.25-2.12 (m)	38.4*	2.25-2.12 (m)	35.7*
12		206.8		206.8		207.8		207.8
13		149.5		150.1		152.3		153.1
14		139.5		139.7		137.5		138.6
15	8.35 (d, 14.4)	140.5	7.01 (d, 14.4)	140.5	8.79 (d, 15.9)	151.3	7.52 (d, 15.7)	151.2
16	6.97 (dd, 14.4, 15.0)	128.1	7.00 (dd, 14.4, 15.0)	129.5	6.35 (dd, 7.8, 16.0)	132.5	6.45 (dd, 7.5, 15.6)	133.5
17	6.98 (d, 15.0)	148.9	6.96 (d, 15.0)	147.6	9.66 (d, 7.8)	190.6	9.68 (d, 7.5)	191.8
18		139.5		140.4				
19	9.47 (s)	194.9	9.50 (s)	194.6				
Me-4	1.08 (s)	19.9	1.08 (s)	19.9	1.07 (s)	19.8	1.07 (s)	19.8
CH_2OH	3.76 (d, 10.4)	67.8	3.76 (d, 10.4)	67.8	3.72 (d, 10.5)	67.9	3.72 (d, 10.5)	67.9
	3.57 (d, 10.4)		3.57 (d, 10.4)		3.58 (d, 10.5)		3.58 (d, 10.5)	
Me-8	1.37 (s)	24.2	1.39 (s)	25.9	1.36 (s)	24.2	1.42 (s)	26.1
Me-10	1.02 (s)	24.8	1.02 (s)	24.8	1.03 (s)	19.8	1.03 (s)	19.8
Me-14	2.04 (s)	15.9	2.31 (s)	14.4	2.00 (s)	15.9	2.26 (s)	14.4
Me-18	1.87 (s)	9.7	1.91(s)	9.9				

*: may be interchanged with closest values in each epimeric mixture.

Aurorals **1**–**4**, which differ from jaspiferals **5**–**8** by the presence of a primary alcohol group at C-4 position, exhibited better cytotoxic activity on the epidermoid human carcinoma KB cells. Aurorals **1**–**2** and jaspiferals **5**–**6** showed ID_{50} values of 0.2 and 5.5 μg/ml, respectively. Aurorals **3**–**4** showed

a moderate activity on KB cells with an ID_{50} of 8 μg/ml, while jaspiferals **7**–**8** were inactive until 10 μg/ml.

Other closely related isomalabaricane terpenoids were previously isolated from the marine sponges *Jaspis stellifera*,[1–3,5] *Stelletta sp*.,[4] *Stelletta tenuis*,[6] *Stelletta globostellata*[7] and *Rhabdastrella globostellata*[8] suggesting that *Jaspis stellifera* should be reassigned as *Rhabdastrella sp*.[9]

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