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New additional triterpenoids from the Mediterranean sponge *Raspaciona aculeata*

Maria Letizia Ciavatta,* Gennaro Scognamiglio, Enrico Trivellone, Tiziana Bisogno and Guido Cimino

Istituto per la Chimica di Molecole di Interesse Biologico, Via Campi Flegrei, 34 (comprensorio Olivetti Fabbr. 70), 80078 Pozzuoli Naples, Italy

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Abstract—Here we reported the finding of eight additional raspacionins (**13**–**20**) from the Mediterranean sponge *Raspaciona aculeata*. The structure determination was obtained by spectroscopic techniques, mainly by 1D and 2D nuclear magnetic resonance. We performed biological tests as antifeedant and ichthyotoxicity assays; moreover we reported some preliminary cytotoxicity test with MCF-7 tumoral cell line.

1. Introduction

Triterpenoids are quite rare in the marine environment: known examples are represented by the metabolites isolated from the red algae (in particular genus *Laurencia*), sponges and few molluscs.[1]

Aside from squalene isolated in 1981, the first triterpene[2] was isolated from the Red Sea sponge *Siphonochalina siphonella* dated back to 1983. From then on, only few sponges demonstrated to contain triterpenoids: *Axinella weltneri* from the Indo-Pacific, *Ptilocaulis spiculifer* from the Red Sea, both processed by Kashman's group,[3–7] and the Mediterranean *Raspaciona aculeata* studied by our group.[8–13] Recently, collections of the sponge *S. siphonella*[14] from two different sites gave new triterpenoids with the same carbon skeleton but with different functionalizations.

Here we report the results obtained studying a new collection of *R. aculeata*, that afforded eight novel raspacionins characterized by the same skeleton of those found (**1**–**12**) in the previous collections, but with different functionalizations at C-4, C-10, C-15 and C-21. For the first time the sponge was collected in Italy, along the Sicilian coasts, whereas all the previous samples were from Blanes, northeast of Spain. The Italian sample was massive and easy to cut, whereas those from Spain were every time encrusting.

The new raspacionins were also tested for their antifeedant and ichthyotoxic properties against *Carassius auratus* and *Gambusia affinis*, respectively, and some of them for the cytotoxicity against the tumoral cell line MCF-7.

2. Results and discussion

The lipid-soluble extract from *R. aculeata* was submitted to a Sephadex column yielding several fractions that were purified, first by SiO_2 gel chromatography and subsequently by HPLC, giving eight new compounds **13**–**20**, besides some of the known raspacionins, **1**–**12**, previously reported in the literature. In this paper we name the new compounds from **13** to **20** following the numbering assigned in the previous papers.[13]

The structural characterization of the new triterpenoids is reported starting from compound **13**. Then the other triterpenoids are described adopting an order of selection suggested by the structural analogy with the last described compound. Compound **13** immediately appeared closely related to raspacionin (**1**), the main metabolite of *R. aculeata*.[8] In fact, both compounds possess an identical left half of the molecule.

Compound **13**, 21-oxo-raspacionin, was an optically active compound ($[\alpha]_D=-21.9$) with elemental composition $C_{32}H_{52}O_6$ determined by HREIMS on the molecular ion at *m/z* 532.3670.

Comparison of the ^1H and ^{13}C NMR spectra with those of raspacionin (**1**) led to suggest the presence of an exomethylene group at C-10, a carbinolic proton at C-4, an acetoxy group at C-15. Unlike raspacionin (**1**), the absence in the ^1H NMR spectrum of **13** of the acetyl methyl singlet at

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* Corresponding author; e-mail: lety@mclink.it

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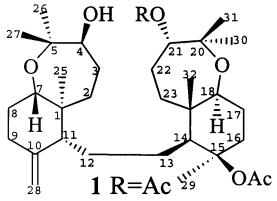

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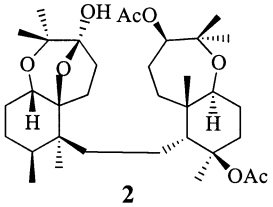

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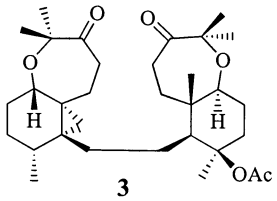

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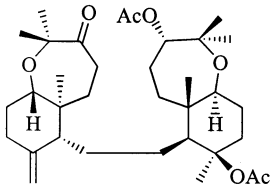

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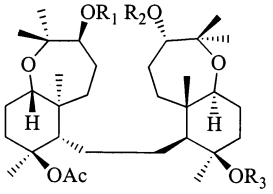

6 R_1=H, R_2=H, R_3=Ac
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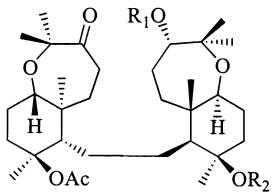

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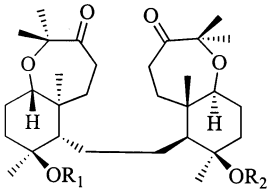

11 R_1=Ac, R_2=H
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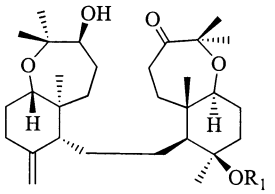
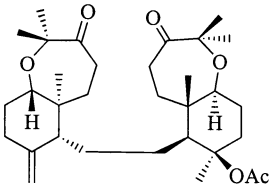
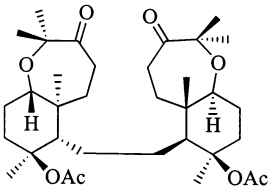


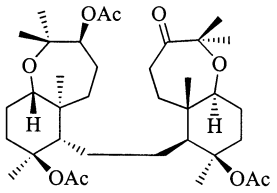
***13** R_1=Ac
***14** R_1=H



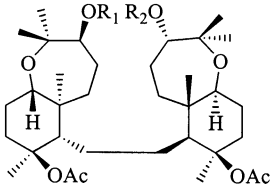
***15**



***16**



***17**



***18** R_1=Ac, R_2=Ac
***19** R_1=H, R_2=Ac
***20** R_1=Ac, R_2=H

* New metabolites isolated from the Thyrrenian *Raspaciona aculeata*

δ 2.17 and of the doublet at δ 4.99, along with the presence of a signal at δ 217.4 in the ^{13}C NMR spectrum, suggested the presence of an oxo group at C-21. This assignment was confirmed by the presence of the downfield signals at δ 3.20–2.12 (H_2-22), completely absent in the spectrum of raspacionin **1**, but present in the ^{1}H NMR spectrum of raspacionin-B (**3**).[11] Furthermore, the resonance of H-18 was upfield (δ 2.96, 3.41 in **1**) shifted by the presence of the oxo group at C-21. All the resonances were assigned by 1D and 2D NMR experiments (DEPT, ^{1}H–^{1}H-COSY, HMQC, HMBC) and reported in Section 3. The relative stereochemistry of **13** was determined by NOESY experiment and by the values of the vicinal coupling constants and appeared similar to that of raspacionin **1**. However, the CD curves showed a positive maximum at 301 nm, thus suggesting an absolute stereochemistry identical to the raspacionins previously reported.[13]

Compound **14** ($[\alpha]_D$=−9.78), 15-deacetyl-21-dioxo-raspacionin, exhibited the formula $C_{30}H_{50}O_5$ suggested by HREIMS on the molecular ion at *m/z* 490.3659.

The ^{1}H NMR spectrum of **14** showed, analogously to that of **13**, signals for one exomethylene, for three carbinolic protons, but for only seven singlet methyls. The main difference was the absence of the signal assigned to the acetoxy group (δ 1.17) at C-15 that was confirmed by the upshifted value of this carbon compared to the same signal in compound **13** (δ 72.5 vs 84.1); moreover, also the carbons nearby were influenced: C-16 (δ 39.0 vs 32.9),

C-29 (δ 30.7 vs 24.7) and C-14 (δ 55.3 vs 57.7). All these data were in full agreement with those previously[13] reported for compound **11**, displaying the same hemistructure.

Compound **15** ($[\alpha]_D$=+8.4), 4,21-dioxo-raspacionin, had molecular formula $C_{32}H_{50}O_6$ deduced by HREIMS spectrum at *m/z* 530.3613. Its ^{1}H NMR spectrum showed signals for an exomethylene, two carbinolic protons, one acetoxy methyl singlet, seven singlet methyls and signals for four protons α to a carbonyl. The two ^{13}C NMR resonances at δ 217.4 and 217.6 suggested the presence of two oxo groups at C-4 and C-21. Of course, ^{1}H and ^{13}C NMR resonances of the atoms belonging to the half bearing the acetoxy group were almost identical to those reported for the corresponding partial structure of **13**, whereas the NMR data of the atoms of the half bearing the exomethylene were in full agreement with those reported for 4-oxo-raspacionin, (**4**)[12] obtained by treating **1** with Jones reagent.

Compound **16**, 10-acetoxy-4,21-dioxo-28-hydroraspacionin, ($[\alpha]_D$=+3.61) had a molecular formula $C_{34}H_{54}O_8$ deduced by HREIMS molecular ion at *m/z* 590.3808. This compound strongly resembled raspacionin **11** and **12**,[13] being made by the left half of **11**, present also in **13**, and the right moiety of **12**. The ^{1}H NMR spectrum exhibited two acetyl methyl groups and signals till δ 3.25 ppm whereas the ^{13}C NMR showed the presence of two signals at δ 217.3 and 217.6 clearly indicating two oxo groups. These signals were located at C-4 and C-21 and were confirmed by the shift of H-18 and H_3-32 besides the expected changes of the nearby atoms.

Compound **17**, 10-acetoxy-4-acetyl-21-oxo-28-hydroraspacionin, ($[\alpha]_D$=−4.44) had molecular formula $C_{36}H_{58}O_9$ deduced by HREIMS molecular ion at *m/z* 634.4083. The ^{1}H NMR spectrum was characterized by three acetoxy groups, a downfield shifted doublet at δ 4.99, five downfield shifted signals between δ 2.5 and 3.5 and eight methyl singlets. On the other hand, ^{13}C NMR showed signals of an oxo group. Although compound **17** showed great similarities with other raspacionins[13] the position of the oxo signal was located at C-21 because influenced the protonic values of the nearby carbons and upshifted H-18 (δ 2.93) and downshifted H_3-32 (δ 1.15). HMBC experiment confirmed this position in particular the presence of the oxo group influenced both C-20 and C-22 that had a long-range correlation with the upfield value of H-18. Of course, compound **17** contains the same left hemistructure present in **15** and **16**, whereas the remaining hemistructure, identical to that of **10**, has been found also in **18**.

Compound **18**, 10-acetoxy-4-acetyl-28-hydroraspacionin, is an optically active compound ($[\alpha_D]$=−19.2) with the molecular formula $C_{38}H_{62}O_{10}$ deduced by HREIMS molecular ion at *m/z* 678.4356 and by ^{1}H and ^{13}C NMR data. The ^{1}H NMR of **18** is very similar to that of raspacionin **10** with the only exception of the acetoxy group linked to C-15: in fact, **18** was characterized by the presence of four acetoxy methyl groups, eight singlet methyls, two methines linked to oxygens and a downshifted doublet integrating for two protons. This latter signal was easily attributed to the methines linked to the secondary acetoxy groups located at C-4 and C-21. The orientation of these substituents was identical to that of raspacionin **10** as suggested by comparison of the NMR data (C-4 δ 78.8, C-21 δ 78.9, **18**; C-4 δ 78.7, C-21 δ 79.0, **10**).[13]

The location at C-10 and C-15 of the other two tertiary acetoxy groups was confirmed also by the downfield protonic shifts of the methyls linked to the same carbons at δ 1.48 and 1.53, respectively.

Furthermore, the ^{13}C NMR resonance of C-28 at δ 19.9 confirmed an axial orientation of this methyl. The equatorial orientation of the acetoxy group at C-10 induced ^{1}H NMR upfield shifts for the equatorial H-9 (δ 2.63; H-16 *eq*. δ 2.82) and for H_3-25 (δ 0.83, H_3-32 δ 0.98) and downfield shifts for C-8 (δ 30.4, C-17 δ 26.4), C-9 (δ 35.5, C-16 δ 33.1) and H-11 (δ 1.52, H-14 δ 0.72).

Compound **19**, 10-acetoxy-28-hydroraspacionin, had an optical rotation of $[\alpha]_D$=−10.6 and a molecular formula of $C_{36}H_{60}O_9$ deduced by HREIMS at *m/z* 636.4244.

Analysis of its ^{1}H NMR spectrum exhibited, besides the eight singlet methyls, three acetoxy methyls, two tertiary and one secondary, one downshifted doublet (δ 4.99) due to the acetyl shift and attributed to H-21, three carbinolic protons. It appeared similar to compound **6** with the only difference at C-21 where an acetoxy group was located instead of a hydroxy group. Proton and carbon resonances appeared quite identical to those of **6** with the exception of the shifts of the nearby carbons produced by the acetoxy group at C-21, that moreover influenced also H-18 (δ 3.41, 3.56 in **6**) and H-14 (δ 0.72, 0.80 in **6**).[13]

Also compound **20**, 10-acetoxy-21-deacetyl-4-acetyl-28-hydroraspacionin, ($[\alpha]_D$=−15.88) exhibited the same molecular formula of **19** (*m/z* 636.4241) and its ^{1}H NMR spectrum appeared closely related to that of **19**: only a shift of H-18 from δ 3.41 to 3.57 led to assign the carbinolic proton at C-21. As in raspacionin **6**, the absence of an acetyl group at C-21 induces, besides the expected shifts for the atoms near C-21, two downshifted values for H-14 (δ 0.80) and H-18 (δ 3.57).

All the new raspacionins that contained an oxo group (**13**–**17**) displayed a positive CD maximum at 301 nm that suggested an absolute stereochemistry identical to those of the 4-oxo-derivative of raspacionin **1**, the 21-oxo-derivative of raspacionin A and raspacionin B.[13]

All the compounds were tested in the ichthyotoxocity test against *G. affinis*[15] resulting toxic at 10 ppm. Also antifeedant test with *C. auratus*[16] resulted positive at 30 μg/cm^2 of commercial fish food. Cytotoxicity test against MCF-7 tumoral cell line showed an inhibition of the 50% of the cell proliferation for concentrations included between 4 and 8 μM.

The Italian *R. aculeata* appeared massive (1.2 kg wet weight) in contrast with the sponge from Spain. Both displayed a substantially identical secondary metabolism, even though the relative ratios were significantly different.

The previous raspacionins were present also in this collection, in particular raspacionin (**1**) that represented the main compound also for this collection and raspacionin-B (**3**), whereas the most polar compounds (**6**–**12**), first isolated from the Spanish samples, are less abundant (as raspacionin **7**) or completely absent. The weight of the new compounds from the Italian samples are included between 9.4×10^{-3} and 1.2×10^{-2} mM.

3. Experimental

3.1. General experimental procedures

NMR spectra were obtained from Bruker AMX-300 and AMX-500 spectrometers (^{1}H, 500.13 MHz; ^{13}C, 125.76 MHz) using as solvent $CDCl_3$. FTIR spectra were recorded with a Biorad FTS-7 instrument. Optical rotations were recorded on a Jasco DIP-370 polarimeter. Cd measurements were carried out on a Jasco J-710 dicograph. HPLC runs were performed on a Waters apparatus equipped with a differential refractometer. Silica gel used for column chromatography and analytical precoated Si gel F254 plates were from Merck (Darmstadt).

3.2. Extraction and isolation of raspacionins

The sponge *R. aculeata* (a voucher specimen is deposited at ICMIB.) was collected by hand off Faro (Messina, Sicily, Italy) during May 1997 and immediately frozen. Extraction of the sponge (1.2 kg wet weight) was made with Me_2CO and the Et_2O-soluble fraction from the water residue was very rich (10 g). Also BuOH extract (5 g) was very abundant but its chromatographic screening did not reveal any correlated derivatives of raspacionins. Part of the Et_2O fraction was submitted to a silica gel column (starting from light petroleum and increasing with diethyl ether) to yield eight fractions in order of polarity. The most interesting fractions have the same R_f of the main compound isolated from *R. aculeata* and every fraction was passed through a small column of silica gel for further purification and the resulting mixtures was submitted to HPLC, using a Kromasil (Akzo Nobel) RP-18 (25 cm×10 mm, particle size 5 μm) column and as eluent system CH_3CN/H_2O from 9:1 to 7:3 (flow rate 5 ml/min), detecting the raspacionins by a differential refractometer.

3.2.1. 21-Deacetyl-21-oxo-raspacionin (13). Colourless oil, R_f (40% light petroleum/diethyl ether) 0.55, $[\alpha]_D=-21.9$ (c=0.12, $CHCl_3$); CD ($c=7.5\times10^{-4}$ M, EtOH) 20° $[\theta]_{301}=+3160$; IR ν_{max} 3468, 2935, 1732, 1715, 1242, 1082, 756 (liquid film, $CHCl_3$) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 4.90 (1H, s, H-28), 4.60 (1H, s, H-28), 3.83 (1H, bd, J=5 Hz, H-4), 3.64 (1H, dd, J=11.5, 4.9 Hz, H-7), 3.20 (1H, ddd, J=14.0, 10.9, 2.3 Hz, H-22), 2.96 (1H, dd, J=11.4, 4.5 Hz, H-18), 2.82 (1H, ddd, J=15.0, 3.5, 3.5 Hz, H-16), 2.24 (1H, bdd, J=11.0, 4.9 Hz, H-9), 2.12 (bdd, J=10.9, 6.4 Hz, H-22), 2.00 (1H, m, H-3), 1.97 (3H, s, CH_3CO-15), 1.95 (1H, m, H-9), 1.88 (1H, m, H-23), 1.82 (1H, m, H-3), 1.72 (1H, m, H-13), 1.65 (1H, m, H-2), 1.63 (1H, m, H-11), 1.62 (2H, m, H-8 and H-12), 1.51 (1H, m, H-17), 1.51 (3H, s, H-25), 1.50 (1H, m, H-12), 1.48 (1H, m, H-2), 1.39 (1H, m, H-17), 1.38 (1H, m, H-8), 1.31 (1H, m, H-16), 1.31 (3H, s, H-31), 1.28 (3H, s, H-26), 1.25 (3H, s, H-30), 1.20 (1H, m, H-23), 1.18 (1H, m, H-13), 1.12 (3H, s, H-27), 1.09 (3H, s, H-32), 0.70 (1H, bs, H-14), 0.69 (3H, s, H-25); δ_C (125 MHz, $CDCl_3$): 218.1 (s, C-21), 170.2 (s, CH_3CO-15), 147.1 (s, C-10), 107.5 (t, C-28), 84.1 (s, C-15), 82.5 (s, C-20), 80.8 (d, C-18), 78.0 (d, C-4), 77.8 (s, C-5), 75.7 (d, C-7), 57.7 (d, C-14), 53.7 (d, C-11), 43.4 (s, C-1), 42.3 (s, C-24), 39.8 (t, C-23), 35.7 (t, C-9), 35.2 (t, C-22), 33.9 (t, C-2), 33.0 (t, C-8), 32.9 (t, C-16), 29.1 (q, C-26), 27.7 (t, C-12), 26.5 (q, C-31), 26.5 (t, C-17), 26.0 (t, C-3), 25.6 (t, C-13), 25.2 (q, C-29), 22.6 (q, CH_3CO-15), 21.3 (q, C-27), 20.5 (q, C-30), 12.1 (q, C-25 and C-32); EIMS m/z 532 [M$^+$] (1), 472 [M−AcOH]$^+$ (5), 454 [M−AcOH–H_2O] (3), 414 [M−AcOH–C_3H_6O]; HREIMS 532.3670 ($C_{32}H_{52}O_6$ requires 532.3764).

3.2.2. 4,21-Dioxo-15-deacetyl-raspacionin (14). Colourless oil, R_f (40% light petroleum/diethyl ether) 0.30, $[\alpha]_D=-9.8$ (c=0.12, $CHCl_3$); CD ($c=7.48\times10^{-4}$ M, EtOH) 20° $[\theta]_{301}=+2407$; IR ν_{max} 3468, 2918, 1714, 1082, 756 (liquid film, $CHCl_3$) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 4.90 (1H, s, H-28), 4.60 (1H, s, H-28), 3.84 (1H, bd, J=5 Hz, H-4), 3.68 (1H, dd, J=11.5, 4.9 Hz, H-7), 3.19 (1H, ddd, J=14.0, 10.9, 2.3 Hz, H-22), 2.93 (1H, dd, J=11.4, 4.5 Hz, H-18), 2.22 (1H, bdd, J=11.0, 4.7 Hz, H-9), 2.10 (1H, bdd, J=10.9, 6.4 Hz, H-22), 2.02 (1H, m, H-3), 1.89 (1H, m, H-17), 1.87 (1H, m, H-23), 1.80 (1H, m, H-3), 1.70 (1H, m, H-13), 1.65 (1H, m, H-16), 1.65 (1H, m, H-12), 1.64 (1H, m, H-11), 1.63 (1H, m, H-2), 1.60 (1H, m, H-8), 1.50 (1H, m, H-16), 1.46 (1H, m, H-2), 1.46 (1H, m, H-12), 1.40 (1H, m, H-17), 1.35 (1H, m, H-8), 1.32 (3H, s, H-31), 1.27 (3H, s, H-26), 1.26 (3H, s, H-30), 1.24 (1H, m, H-23), 1.21 (1H, m, H-13), 1.18 (3H, s. H-29), 1.11 (3H, s, H-27), 1.11 (3H, s, H-32), 0.72 (1H, bs, H-14), 0.68 (3H, s, H-25); δ_C (125 MHz, $CDCl_3$): 217.6 (s, C-21), 147.1 (s, C-10), 107.3 (t, C-28), 82.3 (s, C-20), 81.4 (d, C-18), 77.7 (s, C-5), 76.7 (d, C-4), 75.8 (d, C-7), 72.2 (s, C-15), 55.3 (d, C-14), 53.6 (d, C-11), 43.6 (s, C-1), 42.5 (s, C-24), 39.7 (t, C-23), 39.0 (t, C-16), 35.7 (t, C-9), 35.1 (t, C-22), 33.9 (t, C-2), 33.0 (t, C-8), 30.5 (q, C-29), 29.0 (q, C-26), 27.6 (t, C-12), 26.5 (q, C-31), 26.5 (t, C-17), 25.9 (t, C-13), 25.9 (t, C-3), 21.2 (q, C-27), 20.3 (q, C-30), 12.1 (q, C-25 and C-32); EIMS m/z 490 [M$^+$] (15), 472 [M−H_2O]$^+$ (5), 414 [M−H_2O–C_3H_6O] (15); HREIMS 490.3659 ($C_{30}H_{50}O_5$ requires 490.3658).

3.2.3. 4,21-Dioxo-raspacionin (15). Colourless oil, R_f (40% light petroleum/diethyl ether) 0.85, $[\alpha]_D=+8.4$ (c=0.12, $CHCl_3$); CD ($c=1.07\times10^{-3}$ M, EtOH) 20° $[\theta]_{301}=+6488$, $[\theta]_{211}=+8192$; IR ν_{max} 2938, 1731, 1714, 1242, 1044, 756 (liquid film, $CHCl_3$) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 4.90 (1H, s, H-28), 4.60 (1H, s, H-28), 3.20 (2H, ddd, J=14.0, 10.9, 2.3 Hz, H-3 and H-22), 3.08 (1H, dd, J=11.5, 4.9 Hz, H-7), 2.93 (1H, dd, J=11.4, 4.5 Hz, H-18), 2.80 (1H, ddd, J=15.0, 3.5, 3.5 Hz, H-16), 2.31 (1H, m, H-3), 2.20 (1H, bdd, J=11.0, 4.8 Hz, H-9), 2.12 (2H, bdd, J=10.9, 6.4 Hz, H-22), 1.96 (3H, s, CH_3CO-15), 1.96 (1H, m, H-9), 1.88 (1H, m, H-2), 1.75 (1H, m, H-23), 1.73 (1H, m, H-13), 1.63 (1H, m, H-12), 1.62 (1H, m, H-8), 1.53 (1H, m, H-11), 1.51 (1H, m, H-17), 1.49 (3H, s, H-29), 1.47 (1H, m, H-12), 1.38 (1H, m, H-17), 1.36 (1H, m, H-8), 1.34 (1H, m, H-16), 1.33 (3H, s, H-31), 1.31 (3H, s, H-27), 1.28 (1H, m, H-2), 1.26 (3H, s, H-26), 1.25 (3H, s, H-30), 1.20 (1H, m,

H-13), 1.15 (1H, m, H-23), 1.08 (3H, s, H-32), 0.82 (3H, s, H-25); 0.67 (1H, bs, H-14); δ_C (125 MHz, $CDCl_3$): 217.6 (s, C-21), 217.0 (s, C-4), 170.1 (s, CH_3CO-15), 146.6 (s, C-10), 107.5 (t, C-28), 84.0 (s, C-15), 82.3 (s, C-20), 82.0 (s, C-5), 80.8 (d, C-18), 80.4 (d, C-7), 57.7 (d, C-14), 53.5 (d, C-11), 42.5 (s, C-1), 42.1 (s, C-24), 39.7 (t, C-23), 39.4 (t, C-2), 35.7 (t, C-9), 35.7 (t, C-22), 33.2 (t, C-8), 32.6 (t, C-16), 27.8 (t, C-12), 26.4 (q, C-31 and C-26), 26.4 (t, C-17), 26.0 (t, C-3), 25.4 (t, C-13), 24.8 (q, C-29), 22.5 (q, CH_3CO-15), 20.4 (q, C-27 and C-30), 12.2 (q, C-25), 12.1 (q, C-32); EIMS *m/z* 530 [M^+] (1), 412 [$M-AcOH-C_3H_6O]^+$ (10); HREIMS 530.3613 ($C_{32}H_{50}O_6$ requires 530.3607).

3.2.4. 10-Acetoxy-4,21-dioxo-28-hydroraspacionin (16). Pale yellow oil, R_f (40% light petroleum/diethyl ether) 0.50, $[\alpha]_D=+3.6$ (c=0.12, $CHCl_3$); CD ($c=4.52\times10^{-2}$ M, EtOH) 20° $[\theta]_{301}=+7291$, $[\theta]_{213}=+6090$; IR ν_{max} 2941, 1731, 1715, 1446, 1366, 1248, 1076, 755 (liquid film, $CHCl_3$) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 3.20 (2H, bddd, J=14.0, 10.9, 2.3 Hz, H-3 and H-22), 2.95 (1H, dd, J=11.4, 4.5 Hz, H-18), 2.93 (1H, dd, J=11.0, 4.7 Hz, H-7), 2.84 (1H, ddd, J=15.0, 3.5, 3.5 Hz, H-16), 2.63 (1H, ddd, J=14.8, 3.4, 3.4 Hz, H-9), 2.12 (2H, bdd, J=10.9, 6.4 Hz, H-3 and H-22), 1.95 (3H, s, CH_3CO-15), 1.90 (3H, s, CH_3CO-10), 1.80 (1H, m, H-23), 1.79 (1H, m, H-2), 1.79 (1H, m, H-13), 1.69 (1H, m, H-9), 1.62 (2H, m, H-12), 1.60 (1H, m, H-8), 1.58 (1H, m, H-17), 1.53 (3H, s, H-29), 1.52 (1H, m, H-17), 1.48 (3H, s, H-28), 1.48 (1H, m, H-8), 1.47 (1H, m, H-11), 1.31 (3H, s, H-31), 1.31 (3H, s, H-27), 1.26 (3H, s, H-26), 1.25 (3H, s, H-30), 1.25 (1H, m, H-23), 1.23 (1H, m, H-2), 1.23 (1H, m, H-13), 1.06 (3H, s, H-32), 0.97 (3H, s, H-25); 0.72 (1H, bs, H-14); δ_C (125 MHz, $CDCl_3$): 217.6 (s, C-21), 217.3 (s, C-4), 169.8 (s, CH_3CO-10), 169.7 (CH_3CO-15), 86.4 (s, C-10), 83.6 (s, C-15), 82.7 (s, C-20), 82.6 (s, C-5), 80.8 (d, C-18), 80.5 (d, C-7), 57.7 (d, C-14), 55.5 (d, C-11), 42.1 (s, C-1), 42.0 (s, C-24), 40.4 (t, C-2), 39.9 (t, C-23), 35.2 (t, C-9), 34.9 (t, C-22), 34.9 (t, C-3), 32.8 (t, C-16), 30.2 (t, C-8), 28.9 (t, C-13), 27.9 (t, C-12), 26.4 (q, C-26 and C-30), 26.4 (t, C-17), 24.6 (q, C-29), 22.8 (q, CH_3CO-10), 22.3 (q, CH_3CO-15), 20.4 (q, C-27 and C-31), 19.8 (q, C-28), 12.3 (q, C-25), 12.2 (q, C-32); EIMS *m/z* 590 [M^+] (1), 470 [$M-2AcOH]^+$ (3), 412 [$M-2AcOH-C_3H_6O$] (30); HREIMS 590.3808 ($C_{34}H_{54}O_8$ requires 590.3819).

3.2.5. 10-Acetoxy-4-acetyl-21-oxo-28-hydroraspacionin (17). White oil, R_f (40% light petroleum/diethyl ether) 0.47, $[\alpha]_D=-4.4$ (c=0.12, $CHCl_3$); CD ($c=4.21\times10^{-3}$ M, EtOH) 20° $[\theta]_{301}=+3627$, $[\theta]_{210}=+3325$; IR ν_{max} 2942, 1738, 1731, 1715, 1445, 1366, 1246, 1085, 755 (liquid film, $CHCl_3$) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 4.98 (1H, d, J=7.0 Hz, H-4), 3.45 (1H, dd, J=11.5, 5.0 Hz, H-7), 3.22 (1H, bddd, J=14.0, 10.9, 2.3 Hz, H-22), 2.93 (1H, dd, J=11.4, 4.5 Hz, H-18), 2.85 (1H, ddd, J=15.0, 3.5, 3.5 Hz, H-16), 2.63 (1H, ddd, J=14.8, 3.4, 3.4 Hz, H-9), 2.18 (1H, m, H-22), 2.18 (3H, s, CH_3CO-4), 1.97 (3H, s, CH_3CO-15), 1.93 (3H, s, CH_3CO-10), 1.78 (1H, m, H-3), 1.78 (1H, m, H-13), 1.73 (1H, m, H-9), 1.72 (1H, m, H-23), 1.70 (1H, m, H-3), 1.66 (1H, m, H-12), 1.63 (1H, m, H-17), 1.53 (3H, s, H-29), 1.52 (1H, m, H-11), 1.52 (1H, m, H-8), 1.48 (3H, s, H-28), 1.48 (1H, m, H-17), 1.45 (1H, m, H-8), 1.44 (1H, m, H-2), 1.35 (1H, m, H-2), 1.31 (3H, s, H-27), 1.31 (3H, s, H-31), 1.28 (1H, m, H-16), 1.26 (3H, s, H-26), 1.24 (3H, s, H-30), 1.22 (1H, m, H-12), 1.21 (1H, m, H-13), 1.20 (1H, m, H-23), 1.15 (3H, s, H-32), 0.83 (3H, s, H-25); 0.69 (1H, bs, H-14); δ_C (125 MHz, $CDCl_3$): 217.6 (s, C-21), 170.2 (s, CH_3CO-4), 169.9 (s, CH_3CO-10 and CH_3CO-15), 87.1 (s, C-10), 83.8 (s, C-15), 82.4 (s, C-20), 80.9 (d, C-18), 78.8 (d, C-4), 77.5 (s, C-5), 76.1 (d, C-7), 57.8 (d, C-14), 55.6 (d, C-11), 42.8 (s, C-1), 42.0 (s, C-24), 39.9 (t, C-23), 35.9 (t, C-2), 35.4 (t, C-9), 35.1 (t, C-22), 32.9 (t, C-16), 30.3 (t, C-8), 28.8 (t, C-13), 28.8 (q, C-26), 28.0 (t, C-12), 26.5 (t, C-17), 26.3 (q, C-31), 24.6 (q, C-29), 23.1 (t, C-3), 22.9 (q, CH_3CO-10), 22.4 (q, CH_3CO-15), 21.5 (q, C-27), 21.3 (q, CH_3CO-4), 20.4 (q, C-30), 19.9 (q, C-28), 13.1 (q, C-25), 12.3 (q, C-32); EIMS *m/z* 634 [M^+] (1), 574 [$M-AcOH]^+$ (15), 514 [$M-2AcOH$] (10), 456 [$M-2AcOH-C_3H_6O$]; HREIMS 634.4083 ($C_{36}H_{58}O_9$ requires 634.4081).

3.2.6. 10-Acetoxy-4-acetyl-28-hydroraspacionin (18). Colourless oil, R_f (40% light petroleum/diethyl ether) 0.47, $[\alpha]_D=-19.2$ (c=0.12, $CHCl_3$); CD ($c=1.08\times10^{-3}$ M, EtOH) 20° $[\theta]_{298}=+6.515$; IR ν_{max} 2943, 1731, 1244, 1081, 755 (liquid film, $CHCl_3$) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 5.00 (2H, d, J=7.0 Hz, H-4 and H-21), 3.49 (1H, dd, J=11.5, 5.0 Hz, H-7), 3.43 (1H, dd, J=10.9, 4.7 Hz, H-18), 2.80 (1H, ddd, J=15.0, 3.5, 3.5 Hz, H-16), 2.63 (1H, ddd, J=14.8, 3.4, 3.4 Hz, H-9), 2.18 (3H, s, CH_3CO-4), 2.15 (3H, s, CH_3CO-21), 1.97 (3H, s, CH_3CO-15), 1.93 (3H, s, CH_3CO-10), 1.80 (1H, m, H-22), 1.80 (1H, m, H-3), 1.75 (1H, m, H-3), 1.75 (1H, m, H-13), 1.75 (1H, m, H-22), 1.68 (1H, m, H-23), 1.65 (1H, m, H-9), 1.62 (1H, m, H-12), 1.53 (3H, s, H-29), 1.52 (1H, m, H-11), 1.52 (1H, m, H-8), 1.49 (1H, m, H-17), 1.48 (3H, s, H-28), 1.44 (1H, m, H-2), 1.44 (1H, m, H-8), 1.40 (1H, m, H-17), 1.37 (1H, m, H-2), 1.31 (3H, s, H-27), 1.31 (3H, s, H-31), 1.30 (1H, m, H-16), 1.30 (1H, m, H-23), 1.27 (1H, m, H-12), 1.26 (3H, s, H-26), 1.25 (3H, s, H-30), 1.22 (1H, m, H-13), 0.98 (3H, s, H-32), 0.83 (3H, s, H-25), 0.72 (1H, bs, H-14); δ_C (125 MHz, $CDCl_3$): 170.1 (s, CH_3CO-4 and CH_3CO-21), 169.9 (s, CH_3CO-10 and CH_3CO-15), 87.0 (s, C-10), 84.1 (s, C-15), 78.7 (d, C-4), 77.9 (d, C-21), 77.5 (s, C-5 and C-20), 76.5 (d, C-18), 76.1 (d, C-7), 58.2 (d, C-14), 55.9 (d, C-11), 42.8 (s, C-1), 42.7 (s, C-24), 35.8 (t, C-2), 35.5 (t, C-9), 35.4 (t, C-23), 33.1 (t, C-16), 30.4 (t, C-8), 28.9 (t, C-13), 28.9 (q, C-26), 28.8 (q, C-31), 28.0 (t, C-12), 26.4 (t, C-17), 24.6 (q, C-29), 23.2 (t, C-3 and C-22), 22.9 (q, CH_3CO-10), 22.4 (q, CH_3CO-15), 21.5 (q, C-30), 21.5 (q, CH_3CO-4), 21.3 (q, C-27), 21.2 (q, CH_3CO-21), 19.2 (q, C-28), 13.2 (q, C-25), 12.9 (q, C-32); EIMS *m/z* 678 [M^+] (1), 558 [$M-2AcOH]^+$ (5), 498 [$M-3AcOH$] (3); HREIMS 678.4356 ($C_{38}H_{62}O_{10}$ requires 678.4343).

3.2.7. 10-Acetoxy-28-hydroraspacionin (19). Colourless oil, R_f (40% light petroleum/diethyl ether) 0.37, $[\alpha]_D=-10.6$ (c=0.12, $CHCl_3$); CD ($c=8.91\times10^{-4}$ M, EtOH) 20° $[\theta]_{296}=+6.847$; IR ν_{max} 3467, 2941, 1728, 1366, 1246, 1082, 755 (liquid film, $CHCl_3$) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 4.99 (1H, d, J=7.0 Hz, H-21), 3.83 (1H, bd, J=5 Hz, H-4), 3.64 (1H, dd, J=11.5, 5.0 Hz, H-7), 3.41 (1H, dd, J=10.9, 4.7 Hz, H-18), 2.83 (1H, ddd, J=15.0, 3.5, 3.5 Hz, H-16), 2.66 (1H, ddd, J=14.8, 3.4, 3.4 Hz, H-9), 2.17 (3H, s, CH_3CO-21), 2.03 (1H, m, H-3), 1.98 (1H, m, H-22), 1.94 (3H, s, CH_3CO-10), 1.90 (3H, s, CH_3CO-15), 1.79 (1H, m, H-22), 1.76 (1H, m, H-13), 1.75 (1H, m, H-3), 1.67 (1H, m, H-23), 1.65 (1H, m, H-9), 1.63

(1H, m, H-2), 1.60 (1H, m, H-12), 1.53 (3H, s, H-29), 1.53 (1H, m, H-11), 1.51 (1H, m, H-8), 1.48 (1H, m, H-17), 1.48 (3H, s, H-28), 1.42 (1H, m, H-8), 1.38 (1H, m, H-17), 1.33 (3H, s, H-31), 1.31 (3H, s, H-27), 1.27 (1H, m, H-23), 1.27 (3H, s, H-26), 1.26 (3H, s, H-30), 1.25 (1H, m, H-12), 1.25 (1H, m, H-2), 1.25 (1H, m, H-16), 1.20 (1H, m, H-13), 0.97 (3H, s, H-32), 0.86 (3H, s, H-25), 0.72 (1H, bs, H-14); δ_C (125 MHz, $CDCl_3$): 170.2 (s, CH_3CO-21), 170.0 (s, CH_3CO-15), 169.9 (s, CH_3CO-10), 87.1 (s, C-10), 84.1 (s, C-15), 78.9 (d, C-21), 77.9 (s, C-5 and C-20), 76.9 (d, C-4), 76.5 (d, C-18), 75.7 (d, C-7), 58.2 (d, C-14), 55.2 (d, C-11), 42.9 (s, C-1), 42.7 (s, C-24), 35.4 (t, C-9 and C-23), 34.6 (t, C-2), 33.1 (t, C-16), 30.3 (t, C-8), 29.2 (q, C-31), 28.9 (t, C-13), 28.9 (q, C-26), 28.1 (t, C-12), 26.4 (t, C-17), 24.6 (q, C-29), 23.2 (t, C-22), 22.8 (q, CH_3CO-10), 22.4 (q, CH_3CO-15), 21.4 (q, C-27), 21.3 (q, C-30), 21.3 (q, CH_3CO-21), 19.7 (q, C-28), 13.3 (q, C-25), 12.8 (q, C-32); EIMS *m/z* 636 $[M^+]$ (1), 576 $[M-AcOH]^+$ (5), 558 $[M-AcOH-H_2O]$ (8), 518 $[M-AcOH-C_3H_6O]$; HREIMS 636.4244 ($C_{36}H_{60}O_9$ requires 636.4237).

3.2.8. 10-Acetoxy-21-deacetyl-4-acetyl-28-hydroraspacionin (20). Yellow oil, R_f (40% light petroleum/diethyl ether) 0.39, $[\alpha]_D=-15.8$ (c=0.12, $CHCl_3$); CD ($c=1.36\times10^{-3}$ M, EtOH) 20° [θ]; IR ν_{max} 3447, 2942, 1731, 1365, 1247, 1083, 755 (liquid film, $CHCl_3$) cm^{-1}; δ_H (500 MHz, $CDCl_3$): 4.98 (1H, d, J=7.0 Hz, H-4), 3.83 (1H, bd, J=5 Hz, H-21), 3.57 (1H, dd, J=10.9, 4.7 Hz, H-18), 3.48 (1H, dd, J=11.5, 5.0 Hz, H-7), 2.79 (1H, ddd, J=15.0, 3.5, 3.5 Hz, H-16), 2.62 (1H, ddd, J=14.8, 3.4, 3.4 Hz, H-9), 2.18 (3H, s, CH_3CO-4), 1.99 (1H, m, H-22), 1.94 (3H, s, CH_3CO-10), 1.92 (3H, s, CH_3CO-15), 1.80 (1H, m, H-3), 1.78 (1H, m, H-13), 1.73 (1H, m, H-3), 1.65 (1H, m, H-12), 1.56 (1H, m, H-17), 1.55 (1H, m, H-23), 1.54 (3H, s, H-29), 1.53 (1H, m, H-11), 1.50 (1H, m, H-8), 1.49 (3H, s, H-28), 1.43 (1H, m, H-8), 1.43 (1H, m, H-2), 1.38 (1H, m, H-22), 1.37 (1H, m, H-2), 1.36 (1H, m, H-17), 1.36 (1H, m, H-23), 1.30 (1H, m, H-13), 1.27 (3H, s, H-26), 1.23 (1H, m, H-12), 1.18 (3H, s, H-31), 1.15 (3H, s, H-30), 1.14 (3H, s, H-27), 0.97 (3H, s, H-32), 0.84 (3H, s, H-25), 0.80 (1H, bs, H-14); δ_C (125 MHz, $CDCl_3$): 170.2 (s, CH_3CO-4), 170.0 (s, CH_3CO-15), 169.9 (s, CH_3CO-10), 87.1 (s, C-10), 84.3 (s, C-15), 78.9 (d, C-21 and C-4), 77.9 (s, C-5 and C-20), 76.2 (d, C-18), 76.1 (d, C-7), 58.1 (d, C-14), 55.8 (d, C-11), 42.8 (s, C-1), 42.8 (s, C-24), 35.8 (t, C-2 and C-23), 35.4 (t, C-9), 33.0 (t, C-16), 30.4 (t, C-8), 29.2 (q, C-31), 28.9 (t, C-13), 28.9 (q, C-26), 28.0 (t, C-12), 26.6 (t, C-17), 24.6 (q, C-29), 23.2 (t, C-2 and C-22), 22.9 (q, CH_3CO-10), 22.4 (q, CH_3CO-15), 21.5 (q, C-27), 21.4 (q, C-30), 21.3 (q, CH_3CO-4), 19.9 (q, C-28), 13.1 (q, C-25), 12.9 (q, C-32); EIMS *m/z* 636 $[M^+]$ (1), 576 $[M-AcOH]^+$ (3), 558 $[M-AcOH-H_2O]$ (4), 518 $[M-AcOH-C_3H_6O]$; HREIMS 636.4241 ($C_{36}H_{60}O_9$ requires 636.4237).

3.3. Biological assays

Ichthyotoxicity assays were conduced using mosquito fish, *G. affinis* (Baird and Girard), as described by Gunthorpe and Cameron.[15] The raspacionins were assayed at 1, 5, 10 μg/ml by dissolving the appropriate amount in 0.5 ml of Me_2CO, resulting ichthyotoxic at 10 μg/ml. Antifeedant test were conduced using *C. auratus* and testing all the raspacionins at concentration of 30 $\mu g/cm^2$ of commercial food pellets (Tetramin) that gave positive response.

Cytotoxicity test was performed with mammary carcinoma F-7 (MCF-7), purchased from DSM (Germany), cultured as described by the manufacturer. Cell proliferation assays were carried out in triplicate in 6-well dished containing subconfluent cells at density of about 50,000 cells/well. Compounds or vehicle (ethanol) were administrated 3 h after cell seeding and then daily at each change of medium. Cells were treated with trypsin and counted by a hemocytometer 4 days after the addition of test substances. Cell viability was checked by trypan blue. In MCF-7 all compounds inhibited cell proliferation, the effects expressed as IC50 (as concentration necessary to achieve 50% inhibition of cell proliferation) were for **20**=6 μM, for raspacionin **1**=4 μM and for **21**=8 μM.

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References

1. Fernandez, J. J.; Souto, M. L.; Norte, M. *Nat. Prod. Rep.* **2000**, *17*, 235–246.
2. Carmeli, S.; Kashman, Y. *J. Org. Chem.* **1983**, *48*, 3517.
3. Rudi, A.; Kashman, Y.; Benayahu, Y.; Schleyer, M. *J. Nat. Prod.* **1994**, *57*, 1416.
4. Rudi, A.; Goldberg, I.; Stein, Z.; Kashman, Y.; Benayahu, Y.; Schleyer, M.; Garcia-Gravalos, M. D. *J. Nat. Prod.* **1995**, *58*, 1702.
5. Rudi, A.; Aknin, M.; Gaydou, E. M.; Kashman, Y. *J. Nat. Prod.* **1997**, *60*, 700.
6. Rudi, A.; Yosief, T.; Schleyer, M.; Kashman, Y. *Tetrahedron* **1999**, *55*, 5555.
7. Rudi, A.; Stein, Z.; Goldberg, I.; Yosief, T.; Kashman, Y.; Schleyer, M. *Tetrahedron Lett.* **1998**, *39*, 1445.
8. Cimino, G.; Crispino, A.; Mattia, C. A.; Mazzarella, L.; Puliti, R.; Trivellone, E.; Uriz, M. *Tetrahedron Lett.* **1990**, *31*, 6565.
9. Puliti, R.; Trivellone, E.; Crispino, A.; Cimino, G. *Acta Crystallogr. Sect. C* **1991**, *C47*, 2609.
10. Cimino, G.; Crispino, A.; de Epifanio, R. A.; Madaio, A.; Mattia, C. A.; Mazzarella, L.; Puliti, R.; Trivellone, E.; Uriz, M. *Tetrahedron* **1992**, *48*, 9013.
11. Cimino, G.; Crispino, A.; Madaio, A.; Trivellone, E.; Uriz, M. J. *J. Nat. Prod.* **1993**, *56*, 534.
12. Cimino, G.; de Epifanio, R. A.; Madaio, A.; Puliti, R.; Trivellone, E. *J. Nat. Prod.* **1993**, *56*, 1622.
13. Cimino, G.; Madaio, A.; Trivellone, E.; Uriz, M. J. *J. Nat. Prod.* **1994**, *57*, 784.
14. Kashman, Y.; Yosief, T.; Carmeli, S. *J. Nat. Prod.* **2001**, *64*, 175.
15. Gunthorpe, L.; Cameron, A. M. *Mar. Biol.* **1987**, *94*, 39.
16. Cimino, G.; De Rosa, G.; De Stefano, S.; Sodano, G. *Comp. Biochem. Physiol.* **1982**, *73B*, 471.