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New dimeric phenanthrenoids from the rhizomes of *Juncus acutus*. Structure determination and antialgal activity

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Abstract—In a study of the allelochemical interactions between the wetland plant *Juncus acutus* and microalgae some dimeric dihydrophenanthrenoids have been isolated. The structures have been determined on the basis of their spectroscopic properties and their phytotoxicity was evaluated on *Selenastrum capricornutum*.

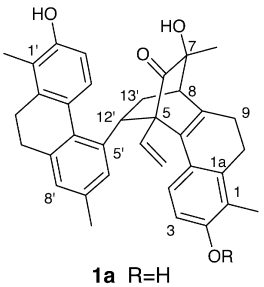
1. Introduction

An extensive study of the aerial parts of *Juncus effusus*[1] and *Juncus acutus*,[2] two wetland plants of the Mediterranean basin led to the isolation of many 9,10-dihydrophenanthrenoids. In vitro assays evidenced the antialgal activity[3] of many of them. In the continuing search for allelochemical interactions between microalgae and macrophytes we have investigated the rhizomes of *J. acutus* and six new 9,10-dihydrophenanthrenes and a dimeric 9,10-dihydrophenanthrenoid have been isolated and characterised.[4,5] Further examination of the ethyl acetate extract of the rhizomes has afforded other five dimeric compounds with significant activity against *Selenastrum capricornutum*, the alga currently used for aquatic toxicity assays.

2. Results and discussion

The ethyl acetate extract was purified by employing a combination of silica gel column chromatography and normal and reversed phase HPLC to afford five new dimers.

Compound **1b** had molecular formula $C_{37}H_{38}O_4$ according to its elemental analysis, the molecular peak at *m/z* 569 $[M+Na]^+$ in a MALDI/MS spectrum, and the presence of 37 carbon signals in the ^{13}C NMR spectrum (Table 1). The ^1H NMR (Table 1) spectrum showed in the aromatic region two coupled doublets at δ 6.99 and 6.55, two coupled doublets at δ 7.30 and 6.73, and two singlets at δ 7.01 and 6.87. Besides three olefinic double doublets at δ 5.79, 5.00 and 4.45 belonging to a vinyl group, a double doublet at δ 4.19, two multiplets of a methylene group at δ 3.18 and 1.91, a methoxyl group at δ 3.79 and four singlets of methyls at δ 2.29, 2.22, 1.98, and 1.50, respectively, were detectable in the aliphatic region. All the remaining protons were present as overlapped signals in the 3.1–2.2 ppm range.



1a R=H
1b R=Me

These data and those of the ^{13}C NMR spectrum and the DEPT experiment closely resembled those of compound **1a**[5] so that the methylether structure **1b** was attributed to this substance. The position of the methoxyl group derived by the NOE correlation between the methoxyl group and the H-3 proton, observed in a 1D-ROESY experiment.

Compound **2** had molecular formula $C_{36}H_{36}O_4$ as established by the elemental analysis and the molecular peak at *m/z* 555 $[M+Na]^+$ in a MALDI/MS spectrum. Its EI/MS spectrum presented peaks at *m/z* 282 and 250 corresponding to $[M-C_{18}H_{18}O]^+$ and $[C_{18}H_{18}O]^+$ attributable to a retro Diels–Alder of the cyclohexene ring. The IR spectrum contained absorption bands at 3790, 3688, 1725 and 1598 cm^{-1}, characteristic of hydroxyl, carbonyl and

Keywords: *Juncus acutus*; dimeric phenanthrenoids; spectroscopic analysis; *Selenastrum capricornutum*; toxicity test.

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Table 1. NMR spectral data of compound **1b** in $CDCl_3$

Position	δ_H[a]	δ_C	Position	δ_H[a]	δ_C
1		120.9 (q)[b]	1′		123.1 (q)
2		156.3 (q)	2′		153.2 (q)
3	6.55 d (8.4)	106.6 (t)	3′	6.73 d (8.4)	111.7 (t)
4	6.99 d (8.4)	126.2 (t)	4′	7.30 d (8.4)	125.9 (t)
5		61.3 (q)	5′		138.5 (q)
6		209.8 (q)	6′	6.87 s	127.5 (t)
7		72.0 (q)	7′		135.8 (q)
8	3.04 m	48.2 (t)	8′	7.01 s	124.6 (t)
9	2.50 m	30.8 (s)	9′	2.40 m	31.7 (s)
10	2.90 m	24.9 (s)	10′	2.80 m	28.4 (s)
1a		140.4 (q)	1a′		137.2 (q)
4a		125.2 (q)	4a′		126.6 (q)
5a		130.0 (q)	5a′		133.4 (q)
8a		140.2 (q)	8a′		141.9 (q)
11	2.22 s	11.6 (p)	11′	2.29 s	11.8 (p)
12	5.79 dd (18.0, 11.6)	135.6 (t)	12′	4.19 dd (8.0, 9.2)	38.4 (t)
13	5.00 dd (11.6, 1.5); 4.45 dd (18.0, 1.5)	117.0 (s)	13′	1.91 m; 3.18 m	34.4 (s)
14	1.50 s	25.9 (p)	14′	1.98 s	21.0 (p)
OMe	3.79 s	55.4 (p)			

[a] ^1H chemical shift values (δ ppm from $SiMe_4$) followed by multiplicity and then the coupling constants (J in Hz).
[b] Letters, p, s, t and q, in parentheses indicate, respectively, the primary, secondary, tertiary and quaternary carbons, assigned by DEPT.

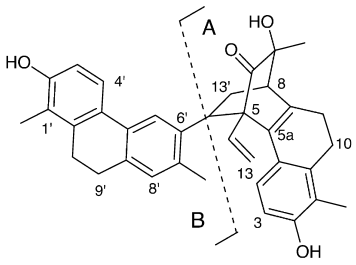
aromatic groups. A close inspection of the ^1H and ^{13}C NMR spectra of **2** (Table 2) by DEPT and HSQC experiments and comparison with the spectral data for the dimer **1a**[5] revealed the presence of the following functionalities: a carbonyl group, two quaternary sp^3-carbons (C-5 and C-7) the last one bearing oxygen, two aliphatic methines (C-8 and C-12′), five aliphatic methylene carbons (C-9, C-10, C-9′, C-10′ and C-13′), four methyls (C-11, C-14, C-11′ and C-14′) a

Table 2. NMR spectral data of compound **2** in $CDCl_3$

Position	δ_H[a]	J (Hz)	$^1H-^1H$ COSY	ROESY	δ_C	HMBC (C)[b]
1					121.4 (q)[c]	
2					152.7 (q)	
3	6.46 d	8.4	4		111.9 (t)	1, 4a
4	7.30 d	8.4	3	12	124.8(t)	2, 1a, 5a
5					58.8 (q)	
6					210.0 (q)	
7					72.3 (q)	
8	2.92 m		13′ *endo*, 13′ *exo*	14	48.4 (t)	6, 7, 5a, 8a, 12′, 13′
9	2.43 m; 2.60 m		10	14	28.2 (s)	10, 5a, 8a
10	2.85 m; 2.63 m		9		25.0 (s)	9, 1a, 4a, 8a
1a					137.3 (q)	
4a					125.9 (q)	
5a					129.3 (q)	
8a					141.8 (q)	
11	2.16 s			10	11.4 (p)	1, 2, 1a
12	6.14 dd	18.5, 11.0	13 *cis*, 13 *trans*	4, 13 *cis*	136.2 (t)	5, 6, 5a, 12′
13	5.25 dd; 5.23 dd	1.5, 11.0, 18.5	12	12, 12′	117.2 (s)	5, 12
14	1.41 s			8, 9	25.3 (p)	6, 7, 8
1′					120.9 (q)	
2′					152.6 (q)	
3′	6.30 d	8.4	4′		112.8 (t)	1′, 4a′
4′	6.38 d	8.4	3′	5′	122.0 (t)	2′, 1a′, 5a′
5′	7.41 s			4′, 13′ *endo*	122.1 (t)	7′, 4a′, 8a′
6′					141.8 (q)	
7′					133.6 (q)	
8′	6.91 s			9′, 14′	129.4 (t)	6′, 5a′
9′	2.93 m; 2.85 m		10′	8′	28.5 (s)	10′, 5a′, 8a′
10′	2.83 m; 2.72 m		9′		25.9 (s)	9′, 1a′, 4a′, 8a′
1a′					137.6 (q)	
4a′					127.7 (q)	
5a′					133.2 (q)	
8a′					133.6 (q)	
11′	2.30 s			10′	11.7 (p)	1′, 2′, 1a′
12′	3.65 dd	10.0, 7.2	13′ *endo*, 13′ *exo*	13′ *exo*, 13, 14′	41.3 (t)	5, 6, 5a, 6′, 7′, 13′
13′	2.94 m; 1.51 m		8, 12′	12′	32.6 (s)	7′, 12′
14′	2.31 s			8′, 12′	19.8 (p)	6′, 7′, 8′

[a] ^1H chemical shift values (δ ppm from $SiMe_4$) followed by multiplicity and then the coupling constants (J in Hz).
[b] HMBC correlations from H to C.
[c] Letters, p, s, t and q, in parentheses indicate, respectively, the primary, secondary, tertiary and quaternary carbons, assigned by DEPT.

methylene (C-13) and a methine (C-12) of a vinyl group, two quaternary sp^2-carbons (C-5a and C-8a), three tetrasubstituted aromatic rings (C-1–C-4, C-1a, C-4a; C-1′–C-4′, C-1a′, C-4a′ and C-5′–C-8′, C-5a′, C-8a′). The connection of these functional groups was determined on the basis of $^1H-^1H$ COSY and HMBC correlations (Table 2), and the planar structure of **2** was elucidated. On the basis of the observed correlations the structure of the tetracyclic moiety (A) was identical to that of compound **1a**. Thus a different connection with the 9,10-dihydrophenanthrene unit (B) was supposed. In the HMBC spectrum the H-12′ proton was heterocorrelated to the C-5 and C-6 of the A unit and to the C-6′ and C-7′ of the B unit. The last two carbons and C-8′ were correlated to the methyl 14′. The singlet at δ 7.41 attributed to H-5′ gave correlations with C-4a′, C-7′ and C-8a′; the first carbon was also correlated with the H-3′ proton. These data indicated a linkage between C-12′ and C-6′. According to the structure the analysis of ROESY spectrum evidenced NOEs of the methyl 14′ with both H-8′ and H-12′.



2

The relative configuration of **2** (Fig. 1) was deduced from detailed analysis of NOEs observed in the ROESY experiments. The minimized structure of **2** obtained by Molecular Mechanics calculations (MM2)[6] was used to predict the NOEs. In the hypothesis of an *S* configuration at C-5 and consequently an *R* configuration at C-8, the *R* configuration at C-7 was supported by the NOE interaction of the H-14 methyl with one of the H-9 protons. The 1,3 spatial interaction[5] of the *exo* H-13′ proton with the hydroxyl group at C-7 caused its downfield shift at 2.94 ppm. The *exo* H-13′ gave NOE with the H-12′ proton, thus justifying their *cis* relation. Both the *endo* H-13′ proton and the H-4′ proton gave NOE with the H-5′ proton. Consequently, the 9,10-dihydrophenanthrene moiety at C-12′ was *endo*-oriented according to the *S* configuration. In conclusion, the absolute configuration of **2** could be 5*S*, 7*R*, 8*R*, 12′*S* or the enantiomeric one.

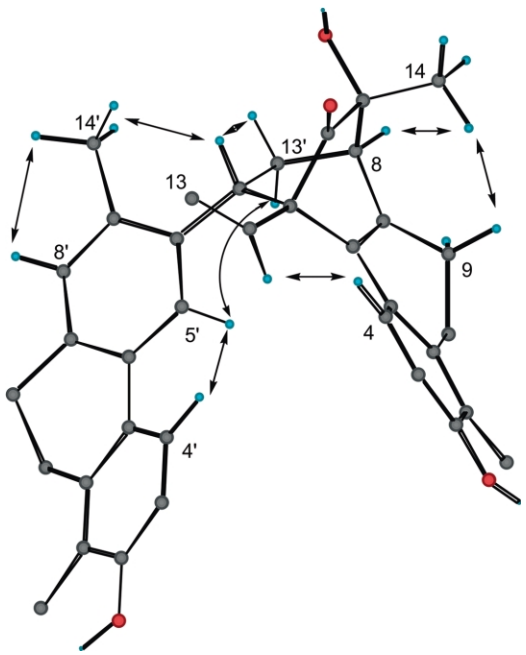
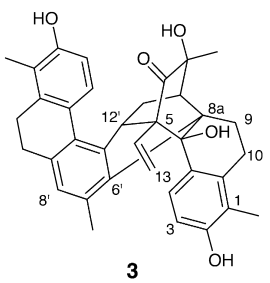


Figure 1. Selected NOE interaction of compound **2**.

Compound **3** had a molecular formula $C_{36}H_{36}O_5$ according to its elemental analysis and the molecular peak at *m/z* 548 $[M]^+$ in a MALDI/MS spectrum, with one oxygen more than **1a**. The ^{13}C NMR spectrum (Table 3) evidenced the presence of two quaternary sp^3-carbons, (C-5a and C-8a) the first one bearing oxygen, instead of two sp^2-carbons as in compound **1a**. The same degree of unsaturations and the presence of one more quaternary aromatic carbon indicated a further ring in the molecule. Further analysis of the ^1H and ^{13}C NMR spectra (Table 3) by DEPT and HSQC experiments revealed the presence of the following functionalities: a carbonyl group, four quaternary sp^3-carbons (C-5, C-5a, C-7 and C-8a), two aliphatic methines (C-8 and C-12′), five aliphatic methylenes (C-9, C-10, C-9′, C-10′ and C-13′), four methyls (C-11, C-14, C-11′ and C-14′), a methylene (C-13) and a methine (C-12) of a vinyl group, two tetrasubstituted aromatic rings (C-1–C-4, C-1a, C-4a; C-1′–C-4′, C-1a′, C-4a′) and one pentasubstituted benzene (C-5′–C-8′, C-5a′, C-8a′). The connection of these functional groups was determined on the basis of $^1H-^1H$ COSY and HMBC correlations (Table 3), and the planar structure of **3** was elucidated. The H-4 proton gave cross peak with the C-5a carbon and, to this carbon, were also correlated the vinylic H-12 proton at δ 5.77, the H-8 at δ 1.72 and the H-12′ at δ 3.93 aliphatic methines. The correlations of the carbon at δ 44.8 with the protons H-8, H-9, H-10 and H-13′ allowed this quaternary carbon to be assigned as C-8a. According to the structure the H-12′ proton was heterocorrelated to the C-5, C-5a and C-12 carbons of the first unit, and to the C-5′, C-6′ and C-13′ carbons of the second one. The analysis of ROESY spectrum (Fig. 2) evidenced NOEs of the H-12′ proton with H-4′ and H-13 *trans* protons, the methyl 14′ with H-8′ and H-9 at δ 2.88 protons and the methyl 14 with hydroxyl at δ 3.85 and H-9 proton at δ 2.69. These data confirmed the structure of dimer **3**. It could be derived from **1a** by formation of a linkage between the C-8a and C-6′ carbons and introduction of a hydroxyl at C-5a.



3

The elemental analysis, molecular peak at *m/z* 555 $[M+Na]^+$ in a MALDI/MS spectrum and the presence of

Table 3. NMR spectral data of compound **3** in $CDCl_3$

Position	δ_H[a]	J (Hz)	$^1H-^1H$ COSY	ROESY	δ_C	HMBC (C)[b]
1					121.5 (q)[c]	
2					152.0 (q)	
3	6.48 d	8.5	4		112.2 (t)	1, 4, 4a
4	6.83 d	8.5	3	12	129.8 (t)	1a, 5a
5					38.7 (q)	
6					213.0 (q)	
7					68.6 (q)	
8	1.72 d br	4.5	13′ *endo*, 13′ *exo*		38.1 (t)	5a, 8a
9	2.69 m; 2.88 m		10	14, 14′	26.3(s)	8a
10	2.51 m; 1.64 m		9		24.8 (s)	8a
1a					137.5 (q)	
4a					127.0 (q)	
5a					61.8 (q)	
8a					44.8 (q)	
11	2.16 s				11.3 (p)	1, 2, 1a
12	5.77 dd	18.0, 11.0	13 *cis*, 13 *trans*	4	136.8 (t)	12′, 5a
13	5.12 dd; 4.73 dd	1.1, 11.0, 18.0	12	12′	117.2 (s)	5, 12
14	1.36 s			OH, 9	9.8 (p)	6, 8, 8a
1′					121.2 (q)	
2′					152.7 (q)	
3′	6.70 d	8.5	4′		112.3 (t)	1′, 4a′
4′	7.35 d	8.5	3′	12′, 13′ *endo*	124.7 (t)	1a′, 5a′
5′					145.7 (q)	
6′					142.9 (q)	
7′					133.1 (q)	
8′	6.81 s			14′	130.0 (t)	5a′, 6′
9′	2.73 m; 2.82 m		10′		29.7 (s)	
10′	2.96 m; 2.58 m		9′		25.8 (s)	
1a′					140.0 (q)	
4a′					128.2 (q)	
5a′					127.3 (q)	
8a′					137.4 (q)	
11′	2.27 s			OH, 10′	11.7 (p)	1′, 2′, 1a′
12′	3.93 d	8.0	13′ *exo*	4′, 13′ *exo*, 13 *trans*	47.5 (t)	5, 5a, 12, 5′, 6′, 13′
13′	2.47 m; 2.19 m		8, 12′		26.3 (s)	8a, 5′, 12′
14′	1.58 s			8′, 9	19.4 (p)	6′, 7′, 8′

[a] ^1H chemical shift values (δ ppm from $SiMe_4$) followed by multiplicity and then the coupling constants (J in Hz).
[b] HMBC correlations from H to C.
[c] Letters, p, s, t and q, in parentheses indicate, respectively, the primary, secondary, tertiary and quaternary carbons, assigned by DEPT.

36 carbon signals in the ^{13}C NMR spectrum (Table 4) justified the molecular formula $C_{36}H_{36}O_4$ for the compound **4**. In the IR spectrum were present hydroxyl absorptions at 3806 and 3691, and the carbonyl absorption at 1720 cm^{-1}.

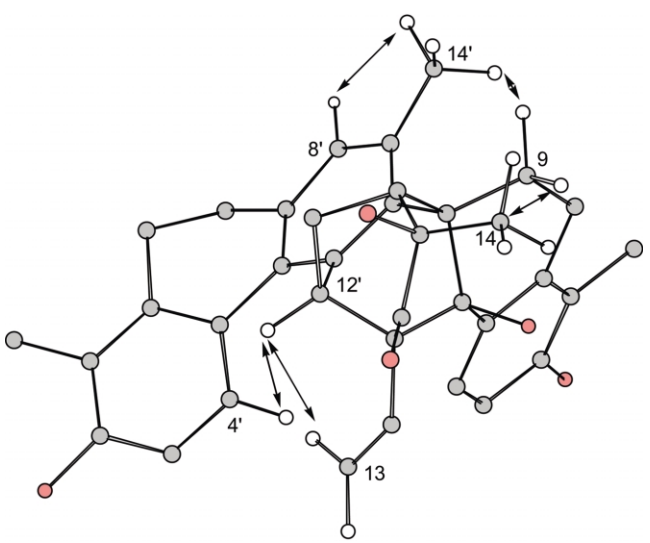


Figure 2. Selected NOE interaction of compound **3**.

The UV spectrum exhibited a maximum at 280 nm. A close inspection of the ^1H and ^{13}C NMR spectra of **4** (Table 4) by DEPT and HSQC experiments revealed the presence of the following functionalities: a carbonyl group, two quaternary sp^3-carbons (C-1 and C-1a) the last one bearing oxygen, two aliphatic methines (C-3 and C-12′), five aliphatic methylenes (C-9, C-10, C-9′, C-10′ and C-13′), four methyls (C-11, C-14, C-11′ and C-14′), a methylene (C-13) and a methine (C-12) of a vinyl group, one tertiary sp^2-carbon (C-4) and one quaternary sp^2-carbon (C-4a), one pentasubstituted and two tetrasubstituted aromatic rings (C-5–C-8, C-5a, C-8a; C-1′–C-4′, C-1a′, C-4a′ and C-5′–C-8′, C-5′a, C-8′a). The connection of these functional groups was determined on the basis of $^1H-^1H$ COSY and HMBC correlations (Table 4), and the planar structure of **4** was elucidated. The protons at δ 3.38 and 6.41 were attributed to the H-3 and H-4 owing to the heterocorrelations with the C-2 carbon. The protons at δ 6.43 and 6.64 were attributed to H-3′ and H-4′ on the basis of the heterocorrelations with the C-2′ carbon. The H-4 proton gave also a cross peak with the C-5a carbon and, to this carbon, were also correlated the vinylic H-12 proton and/or the aromatic singlet H-8 at δ 6.66. The H-12′ proton was heterocorrelated to the C-1 and C-4 carbons of the first unit and to the C-5′ and C-7′ carbons of the second one. Finally the C-1 and C-1a carbons gave a cross peak with the

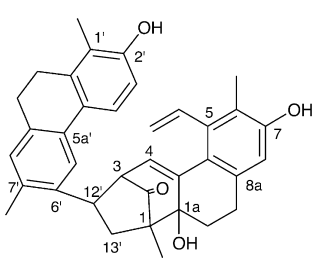
Table 4. NMR spectral data of compound **4** in $CDCl_3$

Position	δ_H[a]	*J* (Hz)	$^1H-^1H$ COSY	ROESY	δ_C	HMBC (C)[b]
1					50.0 (q)[c]	
2					215.0 (q)	
3	3.38 dd	6.8, 2.0	4, 12′	14′	53.4 (t)	1a, 2, 13′
4	6.41 d	6.8	3	12	122.7 (t)	1, 2, 3, 5a
5					138.9 (q)	
6					121.4 (q)	
7					153.6(q)	
8	6.66 s				114.0 (t)	5a, 6
9	2.95 m; 2.78 m		10		28.2 (s)	
10	2.70 m; 2.00 m		9	11	25.6 (s)	
1a					76.1 (q)	
4a					143.4 (q)	
5a					125.6 (q)	
8a					128.2 (q)	
11	1.24 s			10	22.7 (p)	1, 1a, 2
12	6.66 dd	18.1, 11.2	13 *cis*, 13, *trans*	4	137.5(t)	5, 6, 5a
13	5.45 dd; 5.20 dd	2.0, 11.2, 18.1	12	14	120.1 (s)	12
14	2.28 s			13 *trans*	13.7 (p)	5, 6, 7
1′					121.1 (q)	
2′					153.2 (q)	
3′	6.43 d	8.0	4′		113.5 (t)	1′, 2′, 4a′
4′	6.64 d	8.0	3′	5′	122.1 (t)	2′, 1a′, 5a′
5′	7.14 s			4′, 13′ *exo*	122.8 (t)	7′, 8′, 12′
6′					141.2 (q)	
7′					134.3 (q)	
8′	6.96 s			14′	129.8 (t)	6′, 5a′, 8a′, 14′
9′	2.80 m		10′		28.4 (s)	
10′	2.75 m		9′		26.6 (s)	
1a′					137.5 (q)	
4a′					125.6 (q)	
5a′					133.1 (q)	
8a′					140.7 (q)	
11′	2.19 s			10′	11.8 (p)	1′, 1a′, 2′
12′	3.60 m		3, 13′ (*exo*, *endo*)	3, 14′	36.5 (t)	1, 4, 5′, 7′
13′	2.54 dd; 2.02 dd	13.5, 10.0 *endo*, 13.5, 4.7 *exo*	12′	OH, 5′, 12′	32.9 (s)	1, 1a
14′	2.36 s			3, 8′, 12′	19.6 (p)	6′, 7′, 8′

[a] ^1H chemical shift values (δ ppm from $SiMe_4$) followed by multiplicity and then the coupling constants (*J* in Hz).
[b] HMBC correlations from H to C.
[c] Letters, p, s, t and q, in parentheses indicate, respectively, the primary, secondary, tertiary and quaternary carbons, assigned by DEPT.

H-13′ protons. According to the structure the analysis of ROESY (Fig. 3) spectrum evidenced NOEs of the methyl 14′ with H-3, H-8′ and H-12′.

The relative configuration of **4** was attributed on the basis of the protonic couplings and ROESY experiments (Table 4). In the hypothesis of a *R* configuration at C-1 and a *S* one at C-3, the coupling of 2.0 Hz between the H-12′ and the H-3 proton was compatible with an angle of 60°, consequently the 9,10-dihydrophenanthrene group was *exo* oriented according to the *S* configuration at C-12′. To establish the configuration at C-1a the minimized structure obtained by MM2 calculations[6] was used to generate dihedral angles. The values obtained of 5.4 and 121.7° between the H-12′ and H-13′ protons corresponding to couplings of about 8 and 4 Hz, respectively. These values defined the double doublet at δ 2.54 *endo* and the double doublet at δ 2.02 *exo* (Table 4). The NOE between the *endo* H-13′ proton and the hydroxyl proton at δ 4.90 justified their *cis* relation and the *S* configuration at C-1a. In conclusion, the absolute configuration of **4** could be 1*R*, 3*S*, 1*aS*, 12′*S* or the enantiomeric one.



4

Compound **5** had molecular formula $C_{36}H_{32}O_4$ according to its elemental analysis, the molecular ion at *m/z* 528 $[M]^+$ in a MALDI/MS spectrum and the presence of 36 signals in the ^{13}C NMR spectrum (Table 5). In addition to the molecular ion the EI mass spectrum showed significant peaks at *m/z* 265 $[C_{18}H_{17}O_2]^+$ and *m/z* 263 $[C_{18}H_{15}O_2]^+$ corresponding to the 9,10-dihydrophenanthrene and phenanthrene units, respectively. The ^1H NMR spectrum (Table 5) showed the four sharp singlets of the H-11, H-14, H-11′ and H-14′ methyls, the two benzylic multiplets H-9′ and H-10′ methylenes, six double doublets of H-12, H-13 and H-12′, H-13′ the two vinyl groups and the aromatic singlets of the H-4′ and H-8′ protons and the doublets of the H-3, H-4, H-9 and H-10 protons. These data matched those of a phenanthrene and 9,10-dihydrophenanthrene unities. The linkage between the unities was attributed on the basis of HMBC and ROESY experiments. In the HMBC experiment both the H-3 and H-10 protons were correlated to C-4a and

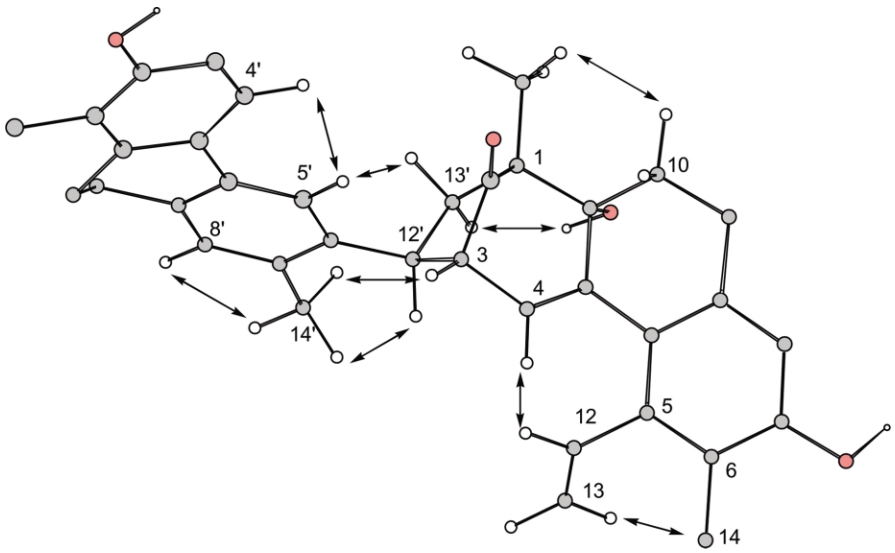


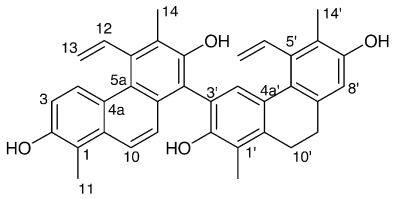
Figure 3. Selected NOE interaction of compound **4**.

C-1 carbons. The H-4 proton gave cross peaks with C-2, C-1a and C-5a carbons. The latter carbon was also correlated with C-12 vinyl carbon. The H-4′ proton was correlated with C-2′, C-1a′, C-5a′ and C-8 carbons. The correlation of the C-8 carbon also with H-9 proton indicated a linkage between C-3′ and C-8 of the unities. According to the structure the analysis of ROESY spectrum evidenced NOEs of the H-11 methyl with the H-10 proton, both the H-14 methyl and the H-4 proton with H-12 proton and that of the H-12′ proton with the H-4′ proton and H-14′ methyl.

Table 5. NMR spectral data of compound **5** in $CDCl_3$

Position	δ_H[a]	*J* (Hz)	$^1H-^1H$ COSY	ROESY	δ_C	HMBC (C)[b]
1					117.0 (q)[c]	
2					150.8 (q)	
3	7.02 d	9.2	4		114.6 (t)	1, 2, 4a
4	8.87 d	9.2	3	12	128.2 (t)	1a, 5a
5					137.9 (q)	
6					125.1 (q)	
7					150.5 (q)	
8					115.2 (q)	
9	7.37 d	9.6	10		123.2 (t)	1a, 5a, 8, 8a
10	7.78 d	9.6	9	11	130.7 (t)	1, 1a, 4a
1a					132.9 (q)	
4a					126.0 (q)	
5a					124.9 (q)	
8a					129.4 (q)	
11	2.56 s			10	12.4 (p)	1, 2, 1a
12	7.32 dd	18.0, 11.2	13 *cis*, 13 *trans*	4, 14	139.7 (t)	5, 6, 13
13	5.76 dd; 5.38 dd	1.6, 11.2, 18.0	12	14	119.5 (s)	5, 12
14	2.52 s			12, 13 *trans*	15.1 (p)	5, 6, 7
1′					121.9 (q)	
2′					150.8 (q)	
3′					115.7 (q)	
4′	7.50 s			12′	124.5 (t)	2′, 1a′, 5a′, 8
5′					137.6 (q)	
6′					120.9 (q)	
7′					152.5 (q)	
8′	6.70 s			9′	113.3 (t)	6′, 5a′, 7′
9′	2.85 m			8′	30.4 (s)	10′, 5a′, 8a′
10′	2.76 m			11′	26.0 (s)	1′, 9′, 4a′
1a′					141.1 (q)	
4a′					128.3 (q)	
5a′					127.2 (q)	
8a′					138.0 (q)	
11′	2.35 s			10′	11.4 (p)	1′, 1a′, 2′
12′	6.83 dd	18.0, 11.2	13′ *cis*, 13′ *trans*	14′, 4′	137.7 (t)	5′, 6′, 5a′
13′	5.36 dd; 5.11 dd	2.0, 11.2, 18.0	12′		119.9 (s)	5′
14′	2.20 s			12′, 13′ *trans*	13.1 (p)	6′, 7′, 8′

[a] ^1H chemical shift values (δ ppm from $SiMe_4$) followed by multiplicity and then the coupling constants (*J* in Hz).
[b] HMBC correlations from H to C.
[c] Letters, p, s, t and q, in parentheses indicate, respectively, the primary, secondary, tertiary and quaternary carbons, assigned by DEPT.



5

The phytotoxicity of all the compounds was tested on the freshwater green alga *S. capricornutum*, using a three-day microbiotest independent of the live culture stocks of the alga. The method measured the response of the algal population in terms of changes in cell density (cell counts per mL) when exposed in static systems to five concentrations for each pure chemical and three replicates, including the controls. The values were reported against $\log_{10}$-transformed data of concentrations (mol/L) to determine the IC_{50}, the concentration estimated to cause a 50% decrease in algal cell density relative to the control, using a linear interpolation method.[7] Confidence limits were calculated by standard deviation and *t* student and the data are reported in Table 6.

The antialgal activities of the dimeric phenanthrenoids were higher than that of 9,10-dihydrophenanthrene.[2] As already observed in monomeric 9,10-dihydrophenanthrene a reduction of the polarity, in related compounds, causes a decrease in the activity. Compound **1b** was about two times less active than **1a**.

3. Experimental

3.1. General procedures

NMR spectra were recorded at 25°C on a Varian UNITY INOVA-500 spectrometer, operating at 500 and 125 MHz for ^1H and ^{13}C, respectively. Matrix assisted laser desorption ionization (MALDI) mass spectra were recorded using a Voyager-DE MALDI-TOF mass spectrometer. EI/MS spectra were recorded with a HP 6890 spectrometer equipped with a MS 5973 N detector. Liquid chromatography over silica gel (230–400 mesh) was performed in a medium pressure. Preparative HPLC was run on a Varian 5500 equipped with an UV detector and using SiO_2 (LiChrospher Silica 10 μm, 250×10 mm i.d., Merck), NH_2 (LiChrospher 100, 10 μm, 250×10 mm i.d., Merck) RP-18 (LiChrospher 10 μm, 250×10 mm i.d., Merck) columns. Analytical TLC was performed on precoated Merck aluminum sheet (DC-Alufolien Kielselgel 60 F_{254}, 0.2 mm) or RP-18 F_{254} plates with 0.2 mm film thickness. Spots were visualized by UV light or by spraying with H_2SO_4–AcOH–H_2O (1:20:4). The plates were then heated for 5 min at 110°C. Prep. TLC was performed on a Merck Kiesegel 60 F_{254} plates, with 0.5 or 1 mm film thickness.

Table 6. Median inhibition concentration (IC_{50}) for *S. capricornutum*

Compound	IC_{50} (μM)
1a	10.3 (8.9–11.7)
1b	21.6 (18.4–25.4)
2	18.3 (17.3–19.5)
3	16.7 (15.3–18.3)
4	17.2 (13.9–26.3)
5	26.4 (23.2–30.0)

95% confidence interval in brackets.

3.2. Plant material, extraction and isolation of metabolites

Rhizomes of *J. acutus* were collected in Italy (Sardinia) during the autumn (October) and identified by Professor Pollio of the Dipartimento di Biologia Vegetale of University Federico II of Napoli. A voucher specimen (HERBNAPY661) is deposited in the herbarium of the University Federico II.

Air-dried rhizomes (2.5 kg) of the plant were extracted with ethyl acetate (9 L) at room temperature for six days. The extract was concentrated under reduced pressure (16.0 g) and subjected to SiO_2 flash column eluted with hexane–AcOEt (9:1) to afford 5 fractions. Fraction 2 (900 mg) was re-chromatographed on SiO_2 flash column eluting with hexane–Et_2O (3:2) to afford 8 fractions. Subfractions 6–8 (30 mg) were purified by SiO_2 HPLC column [hexane–acetone (7:3)], to give **1b** (17 mg); elemental analysis: found: C, 81.1; H, 6.8. $C_{37}H_{38}O_4$ requires: C, 81.3; H 7.0%. Fractions 3–5 (2.6 g) were re-chromatographed on SiO_2 flash column eluting with $CHCl_3$–acetone gradients to afford 5 subfractions. Subfraction 1 eluted with $CHCl_3$–acetone (95:5) (600 mg) was purified by reversed-phase HPLC column [MeOH–MeCN–H_2O (3:1:1)] to give pure **5** (12 mg); elemental analysis: found: C, 82.0; H, 6.3. $C_{36}H_{32}O_4$ requires: C, 81.8; H 6.1%. Subfraction 2 (950 mg), eluted with $CHCl_3$–acetone (9:1), was purified by SiO_2 HPLC column [hexane–acetone (7:3)], to give **2** (14 mg); elemental analysis: found: C, 81.3; H, 6.9. $C_{36}H_{36}O_4$ requires: C, 81.2; H 6.8%. Subfraction 3 (500 mg) was purified by NH_2 column HPLC [hexane–CH_2Cl_2–MeOH (6:3.5:0.5)], to give **3** (8 mg); elemental analysis: found: C, 79.3; H, 6.7. $C_{36}H_{36}O_5$ requires: C, 78.8; H 6.6%. Subfraction 4–5 (200 mg) was re-chromatographed on SiO_2 flash column eluting with $CHCl_3$–AcOEt (1:1) to afford 10 fractions. Subfractions 4–5 (30 mg) were purified by reversed-phase HPLC [MeOH–MeCN–H_2O (4:3:3)], further purification by NH_2 HPLC column [$CHCl_3$–MeOH (98:2)] gave **4** (5 mg); elemental analysis: found: C, 81.0; H, 6.7. $C_{36}H_{36}O_4$ requires: C, 81.2; H 6.8%.

3.3. Algal growth inhibition test

Pure chemicals (99%) were initially dissolved in DMSO (HPLC grade) and then diluted in double-deionized water to make the final stock solns (solvent concentration <0.01% v/v). An additional control was carried out to detect effects of the solvent in spite of the low toxicity of DMSO to algae. The algal test was performed using cells of *S. capricornutum* deimmobilized from beads of alginate according to the standard operational procedures of the test (Algaltoxkit F™, 1996).[8] The alga was inoculated (1×10^4 cells/mL) in 25 mL of test soln. prepared using the OECD medium[9] in five concentrations for each chemical and three replicates per concentration. Under a light intensity of 8.000 lux at 25°C,

the algal growth was monitored by measuring cell numbers at 0 time and every 24 h with an electronic particle dual threshold counter (Coulter Counter Z2, 100 μm capillary) during three day static exposure. Raw data of cells density were processed by a Microsoft Excel 5.0 program tailored for this test. The algal median growth inhibition was estimated integrating the mean values from time 0 to t_{72} h (area under the curve). Inhibition (%) values were reported against log-transformed data of concentration (μM) to evaluate the slope, the trend of regression and the IC_{50} value,[6] reported here.

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