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CYERCENES, NOVEL POLYPROPIONATE PYRONES FROM THE AUTOTOMIZING MEDITERRANEAN MOLLUSC *CYERCE CRISTALLINA*

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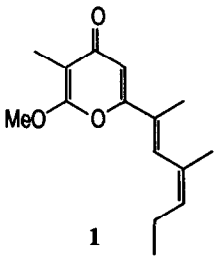
Abstract: Seven novel pyrones of polypropionate biosynthetic origin have been isolated from the dorsal appendages (cerata) of the autotomizing ascoglossan mollusc *Cyerce cristallina*, and their structure established by means of spectroscopic techniques. The new compounds exhibited ichthyotoxic activity. Their possible involvement in the mollusc defensive mechanisms, as well as in regenerative processes following the autotomy, is hypothesized.

Marine gastropod molluscs have always provided interesting models for the study of several aspects of both marine ecology and marine chemistry, biochemistry and physiology. During the last few years we have addressed some of these problems such as chemical defence mechanisms[1-3] as well as correlations between the occurrence of certain secondary metabolites and autotomic defensive behaviour (spontaneous detachment of mantle appendices and/or tail). In particular, chemical studies of the nudibranch *Tethys fimbria* and of the ascoglossan *Oxynoe olivacea*, both exhibiting typical autotomic mechanisms, have recently led to the characterization of both the structure and the origin of novel prostaglandin derivatives, prostaglandin-1,15-lactones[4,5], and of new marine toxins, oxytoxins[6].

Cyerce cristallina is an ascoglossan species whose body volume is mainly due to the presence of dorsal appendages (cerata) aposematically colured in white and red. When attacked by predators this mollusc secretes a supposedly toxic mucus and, if molested further, it detaches its cerata which then exhibit prolonged contractions and carry on secreting large amounts of mucus. The mollusc then regenerates the missing appendages at a very high speed, usually within 7-10 days. Previous chemical studies on species belonging to the genus *Cyerce* are limited, at the best of our knowledge, to the Australian *Cyerce nigricans*[7-8]. Ecological studies have demonstrated that the live mollusc and its crude organic extract are repellent to coral reef fish[7]. However, a recent chemical study, whilst leading to the isolation of the two novel polypropionate pyrones **1** and **2**, did not succeed in identifying the substances responsible for this chemical deterrence[8]. In this paper we report the results of a new study on the chemical composition of the cerata of the Mediterranean *C. cristallina* with the aim of

isolating new secondary metabolites involved in the mollusc chemical defence and, possibly, regenerative processes.



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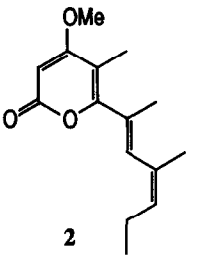

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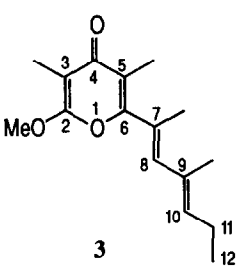
Acetone extraction of *C. cristallina* cerata, followed by diethyl ether extraction and t.l.c. analysis (petroleum ether/ diethyl ether 1/1), yielded five UV visible spots with Rf ranging between 0.1 and 0.6. Preparative t.l.c. followed by HPLC purification led to the isolation of seven pure compounds. These all exhibited strong UV absorptions with λ_{max} ranging between 238 and 356 nm, and IR bands around 1650 and 1720 cm^{-1} typical of γ- and α-pyrones respectively. These observations, together with the previous finding of polypropionate pyrones from *C. nigricans*, suggested a similar structure for the *C. cristallina* metabolites. The seven compounds were divided into three main groups according to their UV, IR, ^1H- and ^{13}C-NMR properties.

The first group of compounds, named cyercenes A and B (**3-4**), consisted of two 2-methoxy-γ-pyrones, as suggested by their IR absorption bands respectively at 1651 and 1655 cm^{-1}, and by the presence of the carbonyl ^{13}C-NMR resonance at δ c. a.181 and of singlets (3H), respectively at δ 3.96 and 4.06, in their $CDCl_3$ ^1H-NMR spectra. In particular, cyercene A ($C_{16}H_{22}O_3$, deduced by HREIMS on the molecular ion peak at *m/z*=262.1561, expected 262.1568) exhibited ^1H- and ^{13}C-NMR data in $CDCl_3$ (Table 1 and 2, respectively) closely related to those reported[9] for the methyl ether of aglajne-3 (**5**). The presence of the 2-methoxy-3,5-dimethyl-γ-pyrone structure was further supported by the comparison of the ^1H-NMR spectra of **3** and **5** in C_6D_6 and by the IR and UV (264 nm) absorptions. The comparison with **5** easily led to the placing on C-6 of an alkyl chain containing an all-*trans* conjugated diene. The relative ^1H-NMR assignments were secured by a series of ^1H-^1H decoupling experiments. The ^{13}C-NMR multiplicities were ascertained by DEPT sequence experiments. The absence of quartets at δ around 22 well fitted with the suggested stereochemistry.



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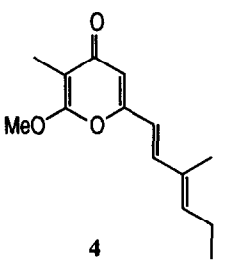

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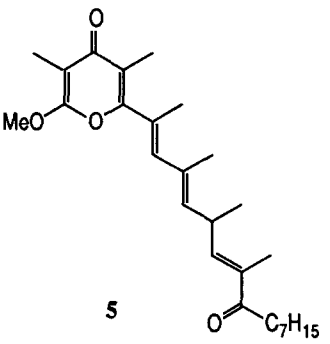

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The second γ-pyrone metabolite, cyercene B (**4**), has a molecular formula ($C_{14}H_{18}O_3$) with only 14 carbon atoms against 16 in **3**. The spectral analysis suggested a structure closely related to **3** but demethylated at

C-5 and C-7. The ^{1}H-NMR spectrum ($CDCl_3$) displayed only 3 signals assignable to methyl groups, e.g. a sharp singlet at δ 1.86, a broad singlet at δ 1.83, which was allylically coupled with a proton resonating at δ 5.80 (H-10), and, finally, a triplet (J=7.5) at δ 1.05 coupled with the protons at δ 2.24 (H-11's). The spectrum was completed by two doublets (J=16.0) at δ 6.90 and 6.03, assignable to the protons of a *trans* oriented disubstituted double bond, and by a sharp singlet at δ 6.12. The latter should be placed on C-5, since irradiation on the methoxy methyl at δ 4.06 did not cause a n.O.e. enhancement of the signal (analogous experiments conducted on the other mono-methyl-methoxy-pyrones isolated from *C. cristallina* **[6, 7, 8, 10, 11]** always resulted in n.O.e. enhancements of the protons on the pyrone rings). The comparison with the ^{13}C-NMR data of the γ-pyrone **1** from *C. nigricans* revealed highly diagnostic signals in the ^{13}C-NMR spectrum of **4**, in particular the quartet at δ 6.7 (CH_3-3) and the doublet at δ 111.4 (H-5). The coupling pattern of H-10, a broad triplet vicinally coupled with the protons at C-11 and allylically coupled with the protons of CH_3-9, easily allowed the definition of the structure of the alkyl chain. The *E* stereochemistry of the trisubstituted double bond was suggested by the high field ^{13}C-NMR value of CH_3-9 (δ=12.0).

TABLE 1

^{1}H-NMR chemical shifts[a], multiplicities and coupling constants (in Hz, in parentheses) for cyercenes, aglajne-3 methyl-ether (**5**) and nectriapyrone (**9**)

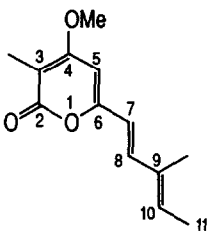
H	**3**	**4**	**6**	**7**	**8**	**10**	**11**	**5**[b]	**9**[10]
3						5.47 *s*	5.47 *s*		
5		6.12 *s*	6.04 *s*	6.04 *s*	6.04 *s*				6.10 *s*
7		6.90 *d* *(16.0)*	7.19 *d* *(15 4)*	7.18 *d* *(15.7)*	7.16 *d* *(15.6)*				
8	6.11 *bs*	6.03 *d* *(16 0)*	5.96 *d* *(15.4)*	5.97 *d* *(15 7)*	5.97 *d* *(15.6)*	6.03 *bs*	6.03 *bs*	6.10 *s*	6.67 *q* *(7.0)*
9									1.75 *d* *(7.0)*
10	5.53 *bt* *(7.5)*	5.80 *bt* *(7 3)*	5.93 *bq* *(7.3)*	5.84 *bt* *(7.4)*	5.67 *bd* *(9.2)*	5.58 *bq* *(6.8)*	5.49 *bt* *(7.2)*	5.38 *d* *(9 0)*	
11	2.16 *m* *(7.5, 7.5)*	2.24 *m* *(7.5, 7.3)*	1.82 *d* *(7.3)*	2.21 *m* *(7.4, 7.5)*	2.67 *m* *(9 2, 6 6)*	1.73 *d* *(6.8)*	2.14 *m* *(7.2, 7 5)*		
12	1.03 *t* *(7.5)*	1.05 *t* *(7.5)*		1.04 *t* *(7.5)*	1.01 *d* *(6.6, 6H)*		1.02 *t* *(7.5)*		
Me-9	1.84 *bs*	1.83 *bs*	1.79 *bs*	1.79 *bs*	1.81 *bs*	1.82 *bs*	1.81 *bs*	1.87 *bs*	
Me-7	2.07 *bs*					2.04 *bs*	2.04 *bs*	2.05 *d* *(1.1)*	1.87 *m* *(1.0)*
Me-5	1.87 *s*					1.94 *s*	1.95 *s*	1.86 *s*	
Me-3	2.01 *s*	1.86 *s*	1.94 *s*	1.94 *s*	1.94 *s*			2.02 *s*	1.90 *s*
O-Me	3.96 *s*	4.06 *s*	3.88 *s*	3.88 *s*	3.88 *s*	3.83 *s*	3.83 *s*	3.96 *s*	3.88 *s*

[a] 500 MHz, $CDCl_3$, TMS = 0

[b] The values are the ones reported in Ref. 9, but the numeration is according to the one reported in this paper.

It is worthwhile noting that cyercene A exhibits a lower λ_{max} (264 nm , ε=14000) than cyercene B (302 nm , ε=11400) in spite of the fact that it contains two more methyl groups. This interesting apparent discrepancy can be explained with the observation that the two methyl groups on C-5 and C-7 of cyercene A do not allow the molecule to assume a planar conformation necessary for the resonance between the γ-pyrone ring and the diene on the alkyl chain to occur, thus lowering the λ_{max}.

Demethylation at the carbons 5 and 7 was also observed in cyercenes 1-3 (**6-8**), all characterized by a 4-methoxy-3-methyl-α-pyrone structure (IR maximum at c.a.1686 cm-1, UV maximum at c.a.350 nm). The ^{1}H-NMR ($CDCl_3$) spectrum of **6** ($C_{13}H_{16}O_3$) showed signals at δ 3.88 (3H, -OCH_3), 1.94 (3H, sharp singlet, CH_3-3), 6.04 (1H, sharp singlet, H-5) assigned to the protons of the α-pyrone ring by comparison with model compounds and, in particular, with nectriapyrone (**9**)[10]. The suggested partial structure was confirmed by a positive n.O.e. observed between the protons of the methoxy group and H-5. The remaining ^{1}H-NMR signals, assigned to the protons of the alkyl chain at C-6, were two coupled doublets (J=15.4) at δ 7.19 and 5.96, a broad singlet at δ 1.79 (CH_3-9) allylically coupled to an olefinic resonance at δ 5.93 which, in turn, was also coupled with a doublet (3H, J=7.3) resonating at δ 1.82. The *E* stereochemistry of the Δ^7 double bond was suggested by the coupling constant (J=15.4) between H-7 and H-8. Scarcity of material prevented a ^{13}C -NMR analysis of cyercene-1, and therefore the stereochemistry of the trisubstituted double bond remains unassigned.



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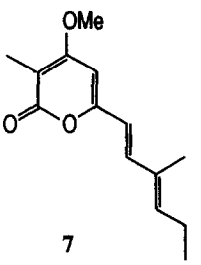

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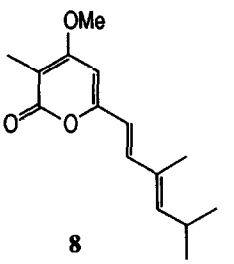

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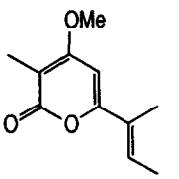

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Cyercene-2 (**7**, $C_{14}H_{18}O_3$) and cyercene-3 (**8**, $C_{15}H_{20}O_3$) exhibited IR (1682, 1693 cm^{-1}) and UV (356 and 353 nm) almost identical to those observed for **6**. In the ^{1}H-NMR spectra ($CDCl_3$) the differences were limited to those expected from the presence, respectively, of one or two additional methyl groups at C-11. Both compounds were also analysed by ^{1}H-NMR in C_6D_6 (Experimental) and ^{13}C-NMR ($CDCl_3$). All the $CDCl_3$ resonances are reported in Tables 1 and 2. The ^{13}C multiplicities were deduced from DEPT sequence experiments. The ^{13}C-NMR signals at δ 11.9 and 12.1, respectively assigned to CH_3-9 of **7** and **8**, suggested an *E* stereochemistry for the trisubstituted double bonds of both compounds.

The UV λ_{max} of cyercenes 1-3 (**6-8**) were in agreement with an extended α-pyrone chromophore and higher than those reported for analogous α-pyrones[9-11], where the resonance between the pyrone ring and the double bonds on the alkyl chain is probably prevented by the presence of methyl groups on C-5 and C-7.

The last two polypropionates from *C. cristallina*, cyercene-4 (**10**) and -5 (**11**), were both characterized by a 4-methoxy-5-methyl-α-pyrone structure. The ^{1}H-NMR spectrum ($CDCl_3$) of cyercene-4 ($C_{14}H_{18}O_3$, IR maximum at 1719 cm^{-1}, UV λ_{max} at 304 nm) displayed signals at δ 5.47 (H-3, sharp singlet), 3.83 (O-CH_3) and 1.94 (CH_3-5, sharp singlet) assigned to the protons of the α-pyrone ring. The demethylation at C-3 was suggested by the strong n.O.e. observed between the protons of the methoxy group and H-3 and further supported by diagnostic ^{13}C-NMR resonance at δ 88.0 (C-3)[12]. The remaining ^{1}H-NMR resonances

were easily assigned to two olefinic protons and three vinyl methyls, arranged on the basis of ^{1}H-^{1}H decoupling experiments in an alkyl chain containing two conjugated double bonds. The *E* stereochemistry of both double bonds was inferred from ^{13}C-NMR evidence showing resonances shifted upfield (δ 16.3 and 16.1) for the carbons of the vinyl methyls at C-7 and C-9. The ^{13}C-NMR multiplicities were assigned by DEPT sequence experiments.

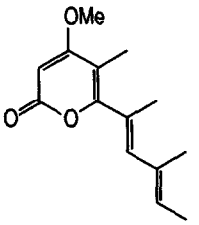
TABLE 2

^{13}C-NMR chemical shifts[a] and multiplicities[b] of cyercenes, aglajne-3 methyl-ether (**5**) and nectriapyrone (**9**)

C	**3**	**4**	**7**	**8**	**10**	**11**	**5**[c]	**9**[10]
2	161.9 *s*	162.3 *s*	165.8 *s**	165.8 *s**	164.1 *s*	n. d.	161.9 *s*	166.4 *s*
3	99.3 *s*	102.5 *s*	102.2 *s*	n. d.	88.0 *d*	88.7 *d*	99.4 *s*	101.6 *s*
4	181.5 *s*	181.0 *s*	164.9 *s**	165.0 *s**	171.4 *s*	n. d.	181.4 *s*	165.4 *s*
5	117.7 *s*	111.4 *d*	94.7 *d*	94.6 *d*	106.4 *s*	n. d.	117.9 *s*	91.8 *d*
6	159.1 *s*	157.2 *s*	158.3 *s*	158.3 *s*	161.8 *s*	n. d.	158.6 *s*	160.3 *s*
7	125.5 *s*	116.5 *d*	116.4 *d*	116.5 *d*	125.8 *s*	n. d.	126.8 *s*	127.2 *s*
8	139.4 *d*	139.5 *d*	140.4 *d**	140.7 *d*	138.9 *d*	138.6 *d*	138.7 *d*	129.5 *d*
9	130.9 *s*	132.4 *s*	132.4 *s*	130.7 *s*	132.5 *s*	n. d.	131.6 *s*	12.1 *d*
10	135.8 *d*	141.4 *d*	141.7 *d**	147.4 *d*	127.9 *d*	135.2 *d*	135.3 *d*	
11	21.6 *t*	22.1 *t*	22.1 *t*	27.9 *d*	13.8 *q*	21.8 *t*		
12	13.8 *q*	13.6 *q*	13.6 *q*	22.5 *q*[e]		14.0 *q*		
Me-9	16.1 *q**	12.0 *q*	11.9 *q*	12.1 *q*	16.1 *q**	16.1 *q**	16.1 *q**	
Me-7	16.3 *q**				16.3 *q**	16.4 *q**	16.9 *q**	14.4 *q*[d]
Me-5	11.8 *q*				11.0 *q*	11.1 *q*	12.0 *q*	
Me-3	6.9 *q*	6.7 *q*	8.7 *q*	8.8 *q*			6.9 *q*	8.6 *q*[d]
OMe	55.2 *q*	55.7 *q*	56.1 *q*	56.1 *q*	56.1 *q*	55.2 *q*	55.3 *q*	56.0 *q*

[a] 125.8 MHz, $CDCl_3$, TMS = 0, some quaternary carbons were not detected (n. d.) for scarcity of material.
[b] Determined by DEPT sequence experiments.
[c] Values are the ones reported in ref. 9, but the numeration is according to the one used here for cyercenes.
[d] These values were interchanged in the original paper[10], but the assignment reported here better fits with model compounds[9,11].
[e] Two signals superimposed.
* Similar assignments with identical superscripts may be interchanged.

The molecular composition ($C_{15}H_{20}O_3$) and the IR (1720 cm^{-1}) and UV (298 nm) maxima immediately led to a structure closely related to **10** for cyercene-5 (**11**). The ^{1}H-NMR spectra ($CDCl_3$ and C_6D_6) confirmed this analogy by displaying signals almost superimposable to those of **10** with the exception of the resonance due to the presence of a further methyl group at C-11. The four sp^3 carbons in the alkyl chain resonated, in the ^{13}C-NMR spectrum, at δ 14.0 (q), 16.1 (q), 16.4 (q) and 21.8 (t). The absence of ^{13}C-NMR quartets around δ 22 excluded a *Z* geometry for the double bonds of **11**.



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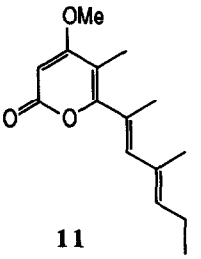

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In agreement with the UV data reported in this study, the λ_{max} of cyercenes -4 and -5 were comparable to the ones obtained by subtracting a value of 15 (because of the absence of a methyl group on C-3) from the values reported for methylated α-pectinatone and the α-pyrone of aglajne-3[9-10]. The values were, however, lower than those observed for cyercenes 1-3 (**6-8**) since, once again, the presence of the methyl groups on C-5 and C-7 does not allow the molecules to assume the planar conformation necessary for maximal electron delocalization.

The limited amount of biological material prevented an extensive 2D-NMR study of cyercenes. However, with the aim of obtaining the clearest possible results, ^{1}H-NMR analysis has been performed by recording spectra in both $CDCl_3$ (Table 1 and 2) and C_6D_6 (Experimental), all the assignments being confirmed by ^{1}H-^{1}H decoupling experiments, while ^{13}C-NMR multiplicities were confirmed by means of DEPT sequence experiments for all compounds except cyercene-1 (**6**).

The polypropionate origin of cyercenes and their *de novo* biosynthesis from ^{14}C-propionic acid was shown by means of *in vivo* absorption incorporation experiments, thus further substantiating the structures presented herein. Cyercenes were also found to have a different distribution within *C. cristallina* mantle, ceras and mucous secretion, and were absent from the mollusc digestive gland, as expected from their *de novo* biosynthesis. The presence in the mucus suggested a defensive role for these metabolites which, indeed, were shown to exert a considerable ichthyotoxic activity on the mosquito fish (*Gambusia affinis*) assay[13] at doses ranging between 5 and 10 μg/ml. Cyercene A (**3**), which was only found in the regenerating tissue of the mollusc, the cerata, exhibited a specific activity in the *Hydra vulgaris* regeneration assay[14]. The biological activity, tissue distribution and biosynthesis of cyercenes will be described in detail in a forthcoming paper[15]. However, the data described herein have led to the structural characterization, from *C. cristallina*, of a novel group of variously demethylated polypropionate pyrones which seem to be, together with the ones described previously[8], specific chemical markers of the ascoglossan genus *Cyerce*. It will be now interesting to assess whether species other than *C. nigricans* and *C. cristallina*, and belonging to the same family (Polybranchiidae), also exhibit this new complex, and possibly multifunctional, secondary metabolic pathway.

Experimental

Extraction and purification of cyercenes

Ten specimens of *C. cristallina* (Trinchese, 1981) were caught in the bay of Naples, and the detachment of their cerata spontaneously induced The cerata (115 mg dry tissue weight), depleted of their mucus, were immediately extracted with acetone and sand (Merck, 1/1 w/w). The acetone extract was then evaporated under vacuum and the aqueous residue extracted three times with diethyl ether. The ethereal extract

(20 mg) was then loaded onto semipreparative t.l.c. plates (Merck), developed with petroleum ether/diethyl ether 1/1, which yielded five UV visible bands at Rf=0.1 (cyercene B, **4**, 1.2 mg), 0.3, 0.45 (cyercene A, **3**, 2.6 mg), 0.55 (cyercene-2, **7**, 1.8 mg) and 0.6 (cyercene-3, **8**, 2.0 mg). These were further purified by means of reverse phase HPLC carried out using a Spherisorb ODS2 column (4.5x250 mm, i.d.=5 μm) eluted with a 40 min gradient from 60 to 75% methanol in water, flow rate 1 ml/min. Thus, the t.l.c. band at Rf=0.3 yielded three HPLC peaks corresponding to cyercene-1 (**6**, 0.7 mg), cyercene-4 (**10**, 3 mg) and cyercene-5 (**11**, 1 mg).

Acquisition of IR, UV, MS and NMR spectra

IR spectra were recorded as thin film on KBr on a Biorad FTS-7 FTIR. UV spectra were obtained in methanol using a DMS-90 Varian spectrophotometer. Electron impact mass spectra were run on Kratos MS30 and MS50 machines. ^{1}H-(500 MHz) NMR spectra were obtained in $CDCl_3$ and C_6D_6, while ^{13}C-(125.8 MHz) NMR analyses were carried out in $CDCl_3$. The instrument used was a Brucker WM 500 MHz spectrometer. HPLC analyses were performed using a Waters Ass. liquid chromatograph equipped with M510 pumps and a 490E multiwavelength UV detector, monitoring UV absorbance at 248 and 285 nm.

Further spectral data of cyercene A (3).

IR ν_{max} at 1651, 1605 and 1585 cm^{-1}. UV λ_{max} at 264 nm (ε=14000). ^{1}H-NMR (C_6D_6, 500 MHz, δ): 5.97 (H-8, 1H, bs), 5.39 (H-10, 1H, bt, J=7.5 Hz), 3.16 (O-CH_3, s), 2.18 (CH_3-5 or CH_3-3, s), 2.17 (CH_3-3 or CH_3-5, s), 1.95 (H-11, 2H, m, J= 7.5, 7.5 Hz), 1.82 (CH_3-7, bs), 1.59 (CH_3-9, bs), 0.89 (H-12, 3H, t, J= 7.5 Hz). EIMS fragments at *m/z*=262 (molecular ion, 55%), 248 (-CH_3, 31%), 234 (-CO 100%) 233 (-C_2H_5, 61%).

Further spectral data of cyercene B (4).

IR ν_{max} at 1655, 1613 and 1566 cm^{-1}. UV λ_{max} at 239 nm (ε=12100) and 302 nm (ε=11400). ^{1}H-NMR (C_6D_6, 500 MHz, δ): 6.66 (H-7, 1H, d, J=16.0 Hz), 6.16 (H-5, 1H, s), 5.68 (H-8, 1H, d, J=16.0 Hz), 5.54 (H-10, 1H, bt, J=7.3 Hz), 3.12 (O-CH_3, s), 2.17 (CH_3-3, s), 1.95 (H-11, 2H, m, J=7.5, 7.3 Hz), 1.47 (CH_3-9, bs), 0.85 (H-12, 3H, t, J=7.5). EIMS fragments at *m/z*=234 (molecular ion, 100%).

Relevant C_6D_6 ^{1}H-NMR data of the methyl ether of aglajne-3 (5).

(500 MHz, δ): 5.95 (H-8, 1H, bs), 5.28 (H-10, 1H, d, J=9.0 Hz), 3.18 (O-CH_3, s), 2.19 (CH_3-3 or CH_3-5, s), 2.18 (CH_3-5 or CH_3-3, s), 1.82 (CH_3-7, bs), 1.58 (CH_3-9, bs).

Further spectral data of cyercene-1 (6).

IR ν_{max} at 1686, 1553 cm^{-1}. UV λ_{max} at 248 nm (ε=16000) and 340 nm (ε=8500). EIMS fragments at m/z=220 (molecular ion, 100%), 205 (-CH_3, 13%), 177 (-CH_3,-CO, 13%).

Further spectral data of cyercene-2 (7).

IR ν_{max} at 1682 and 1548 cm^{-1}. UV λ_{max} at 253 nm (ε=19200) and 356 nm (ε=9400). ^{1}H-NMR (C_6D_6, 500 MHz, δ): 7.13-7.16 (signal obscured by C_6H_6 signal, H-7), 5.69 (H-8, 1H, d, J=15.7 Hz), 5.46 (H-10, 1H, t, J=7.4 Hz), 5.26 (H-5, 1H, s), 2.96 (O-CH_3, s), 2.16 (CH_3-3, s), 1.94 (H-11, 2H, m, J=7.5, 7.4 Hz), 1.59 (CH_3-9, bs), 0.84 (H-12, 3H, t, J=7.4 Hz). EIMS fragments at *m/z*=234 (molecular ion, 100%).

Further spectral data of cyercene-3 (8).

IR ν_{max} at 1693 and 1560 cm^{-1}. UV λ_{max} at 252 nm (ε=15000) and 353 (ε=7960). ^{1}H-NMR (C_6D_6, 500 MHz, δ): 7.13-7.16 (signal obscured by C_6H_6 signal, H-7), 5.68 (H-8, 1H, d, J=15.6 Hz), 5.31 (H-10, 1H, bd, J=9.2 Hz), 5.26 (H-5, 1H, s), 2.96 (O-CH_3, s), 2.46 (H-11, 1H, m, J=9.2, 6.6 Hz), 2.15 (CH_3-3, s), 1.61 (CH_3-9, bs), 0.87 (H-12, 6H, d, J=6.6 Hz). EIMS fragments at *m/z*=248 (molecular ion, 100%), 233 (-CH_3, 19%), 220 (-CO, 8%), 205 (-$CH(CH_3)_2$, 20%).

Further spectral data of cyercene-4 (10).

IR ν_{max} at 1719 and 1710 cm^{-1}. UV λ_{max} at 238 nm (ε=8900) and 304 nm (ε=10200). ^{1}H-NMR (C_6D_6, 500 MHz, δ): 5.89 (H-8, 1H, bs), 5.40 (H-10, 1H, bq, J=6.8), 5.21 (H-3, 1H, s), 2.89 (O-CH_3, s), 1.90 (CH_3-7, bs), 1.75 (CH_3-5, s), 1.57 (CH_3-9, bs), 1.49 (H-11, 3H, d, J=6.8 Hz). EIMS fragments at *m/z*=234 (molecular ion, 100%), 219 (-CH_3, 92%), 205 (-CH_2-CH_3, 29%), 191 (-CO, -CH_3, 54%).

Further spectral data of cyercene-5 (11).

IR ν_{max} at 1720, 1630 cm^{-1}. UV λ_{max} at 240 nm (ε=7500) and 298 nm (ε=8800). ^{1}H-NMR (C_6D_6, 500 MHz, δ): 5.90 (H-8, 1H, bs), 5.38 (H-10, 1H, t, J=7.2 Hz), 5.23 (H-3, 1H, s), 2.88 (O-CH_3, s), 1.96 (H-11, 2H, m, J=7.2, 7.5 Hz), 1.92 (CH_3-7, bs), 1.74 (CH_3-5, s), 1.56 (CH_3-9, bs), 0.88 (H-12, 3H, t, J=7.5 Hz). EIMS fragments at *m/z*=248 (molecular ion, 100%), 233 (-CH_3, 55%), 219 (-CH_2-CH_3, 45%), 205 (-CO, -CH_3, 75%).

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References

1. Cimino, G., De Rosa, S., De Stefano, S., Sodano, G. and Villani, G., *Science*, **1983**, *219*, 1237-1238.
2. Cimino, G., De Rosa, S., De Stefano, S. and Sodano, G., *Pure Appl. Chem.*, **1986**, *58*, 375-386.
3. Cimino, G. and Sodano, G., *Chemica Scripta*, **1989**, *29*, 389-394.
4. Cimino, G., Spinella, A. and Sodano, G., *Tetrahedron Lett.*, **1989**, *30*, 3589-3592.
5. Cimino, G., Crispino, A., Di Marzo, V., Sodano, G., Spinella, A and Villani, G., *Experientia*, **1991**, *47*, 56-61.
6. Cimino, G., Crispino, A., Di Marzo, V., Gavagnin, M. and Ros, J.D., *Experientia*, **1990**, *46*, 767-770.
7. Hay, M.E., Pawlik, J.R., Duffy, J.E. and Fenical, W., *Oecologia*, **1989**, *81*, 418-427.
8. Roussis, V., Pawlik, J.R., Hay, M.E. and Fenical, W., *Experientia*, **1990**, *46*, 327-329.
9. Cimino, G., Sodano, G. and Spinella, A., *J. Org Chem.*, **1987**, *52*, 5326-5331.
10. Nair, M.S.R. and Carey, S.T., *Tetrahedron Lett.*, **1975**, *19*, 1655-1658
11. Biskupiak, J.E. and Ireland, C.M., *Tetrahedron Lett.*, **1983**, *24*, 3055-3058.
12. Turner, W.V. and Pirkle, W.H., *J. Org. Chem.*, **1974**, *39*, 1935-1937.
13. Gunthorpe, L. and Cameron, A.M., *Mar. Biol.*, **1987**, *94*, 39-43
14. De Petrocellis, L., Maharajan, V., De Petrocellis, B. and Minei, R., *J. Embriol. Exp. Morph.*, **1986**, *93*, 105-119.
15. Di Marzo, V., Vardaro, R.R., De Petrocellis, L., Villani, G., Minei, R. and Cimino, G., *Experientia*, submitted.