Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron Letters
Tetrahedron Letters 46 (2005) 2845–2847

Cymodienol and cymodiene: new cytotoxic diarylheptanoids from the sea grass *Cymodocea nodosa*

Ioanna Kontiza,[a] Constantinos Vagias,[a] Jasmin Jakupovic,[b] Dimitri Moreau,[c] Christos Roussakis[c] and Vassilios Roussis[a,*]

[a]*University of Athens, School of Pharmacy, Department of Pharmacognosy and Chemistry of Natural Products, Panepistimiopolis Zografou, Athens 157 71, Greece*
[b]*AnalytiCon Discovery GmbH, Hermannswerder Haus 17, 14473 Potsdam, Germany*
[c]*ISOMer, Laboratoire de Pharmacogénomique Marine, Faculté de Pharmacie, 1 Rue Gaston Veil, F-44035 Nantes, France*

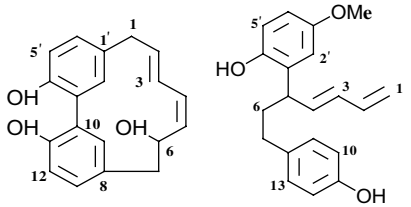
Received 27 December 2004; revised 16 February 2005; accepted 22 February 2005

Abstract—The two new diarylheptanoids, cymodienol (**1**), and cymodiene (**2**), obtained from specimens of the sea grass *Cymodocea nodosa*, collected from the coastal areas of central Greece, are the first members of this class isolated from marine organisms. The chemical structures of the two metabolites were assigned on the basis of their NMR and MS spectroscopic data, including information obtained by 1D- and 2D-NMR experiments. Metabolite **2** possesses an unprecedented skeleton that might be biosynthetically related to metabolite **1**. Cymodienol (**1**) was found to exhibit significant cytotoxic activity against two lung cancer cell lines.

Even though marine phanerogams hold an important ecological role in the global marine ecosystem and numerous studies on the biomass seasonal variation and growth plasticity[1,2] of *Cymodocea nodosa* have been reported, information of its chemical content is very limited.[3,4] In the course of our studies toward the isolation of bioactive metabolites from marine organisms,[5–7] we recently were able to collect and analyze specimens of *C. nodosa* from the sandy marine fields of Ag. Cosmas near Athens.

We herein report the isolation, structure elucidation, and antiproliferative activity of two new biphenyl compounds named cymodienol (**1**) and cymodiene (**2**) from *C. nodosa* that represent the first diarylheptanoids isolated from marine organisms.

Freeze dried *C. nodosa* (489.7 g) was exhaustingly extracted with a mixture of dichloromethane–methanol (3:1) and the brownish oily extract (14.1 g) was subsequently fractionated with a combination of chromatographic techniques to allow, after final HPLC purification, the isolation of (**1**) (4.3 mg) and (**2**) (3.5 mg) (Fig. 1).



Cymodienol (**1**) Cymodiene (**2**)

Figure 1. Structures of cymodienol (**1**) and cymodiene (**2**).

Compound **1** was isolated as a greenish oil with $[\alpha]_D$ −181 (*c* 0.5, CH_2Cl_2).[8] The $[M]^+$ ion at *m/z* 294.1221 in combination with the ^{13}C NMR data required a molecular formula of $C_{19}H_{18}O_3$. The IR and UV spectra revealed the presence of hydroxyl groups and benzene rings.[9,10] The presence of 16 aromatic/olefinic carbons in the ^{13}C NMR spectrum in combination with eleven degrees of unsaturation suggested a tricyclic structure. The multiplicities of the ^{13}C NMR signals were deduced from DEPT spectra which revealed the presence of two methylene, eleven methine, and six quaternary carbons. The benzylic methylene protons at δ 3.02/2.88 were coupled with an oxygenated methine

Keywords: *Cymodocea nodosa*; Diarylheptanoids; Cymodienol; Cymodiene.
* Corresponding author. Tel./fax: +30 210 7274 592; e-mail: roussis@pharm.uoa.gr

0040-4039/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2005.02.123

proton at δ 4.96 which itself correlated with the first proton of a diene system at δ 5.37. The spin system of the conjugated double bonds was clearly resolved by the H–H COSY, additionally showing the coupling of the last proton with the second benzylic methylene. The *Z*, *E* geometry of the double bonds was proposed on the basis of the coupling constants (10.9, 15.6 Hz). Analysis of the ^{1}H NMR and 2D-heteronuclear experiments revealed two 1,2,4-aromatic spin systems for the biphenyl system. The observed long range heteronuclear correlation (see Table 1) and the ^{13}C-chemical shifts of aromatic carbons and the spectral similarities with metabolite alnusdiol previously isolated from the tree *Alnus japonica*,[11] secured the arrangement of the substituents on the aromatic rings. On the basis of the above evidence the structure of a diarylheptanoid possessing a conjugated diene system was assigned for metabolite **1**, and named cymodienol.

Metabolite **2** was obtained as a colorless oil exhibiting an $[\alpha]_D$ −17.0 (*c* 0.1, CH_2Cl_2).[12] The molecular formula as deduced from the FAB-MS (*m/z* 310.1569 $[M]^+$) and ^{13}C NMR data was $C_{20}H_{22}O_3$. Seven overlapping aromatic protons along with five olefinic protons could be detected in the spectrum measured in $CDCl_3$. When the spectrum was measured in a mixture of $CDCl_3$–C_6D_6 (70:30), the upfield shift of the aromatic protons led to a much better resolved spin system. Two doublets at δ 6.83 and 6.45 (8.3) each integrating for two protons, indicated a *para*-substituted phenyl ring. The remaining aromatic signals required 1,2,4-trisubstitution for the second aromatic ring (see Table 1). Two exchangeable signals appearing as broad singlets at δ 3.84 and 3.87 along with a singlet integrating for three protons at δ 3.37 were assigned to two aromatic hydroxyls and an aromatic methoxyl. Evident in the ^{1}H NMR spectrum was the presence of a vinyl group which by H–H COSY was shown to be a part of a conjugated diene system. The *E* configuration of the second double bond was deduced from the coupling constant of 15.3 Hz. The sequence was prolonged to a benzylic methine at δ 3.64 and further through a methylene at δ 1.95 to a second benzylic methylene at δ 2.44. The heteronuclear correlation of the benzylic carbon with the aromatic proton at δ 6.83 secured the position of the trisubstituted phenyl ring of metabolite **2**, named cymodiene. The strong NOE effects between the methoxyl singlet and the *meta* coupled aromatic protons at δ 6.77 (d) and 6.52 (dd) fixed the positions of the hydroxyl and methoxy moieties on the second aromatic ring. All

Table 1. ^{1}H and ^{13}C NMR data (400 and 50.3 MHz, respectively) for metabolites **1** and **2**

	Metabolite **1**				Metabolite **2**			
	^{1}H (δ)[a]	^{13}C (δ)	HMBC		^{1}H (δ)[a]	^{1}H (δ)[b]	^{13}C (δ)	HMBC
H-1a	3.14 (dd, 6.3, 2.9)	35.9	C-3	H-1a	5.12 (d, 17.1)	4.99 (d, 17.1)	116.4	C-2, C-3
H-1b	3.46 (dd, 9.4, 2.9)		C-6′, C-2	H-1b	5.00 (d, 10.3)	4.90 (d, 9.8)		C-3
H-2	5.97 (ddd, 15.6, 9.4, 6.3)	139.1	C-4	H-2	6.31 (ddd, 17.1, 10.3, 10.2)	6.25 (ddd, 17.1, 10.4, 9.8)	136.7	C-3
H-3	6.74 (dd, 15.6, 10.9)	127.4	C-5	H-3	6.10 (dd, 12.7, 10.3)	6.08 (dd, 15.3, 10.4)	131.3	C-4, C-2
H-4	6.05 (dd, 10.9, 10.9)	129.4	C-6, C-2	H-4	5.83 (dd, 12.7, 7.3)	5.75 (dd, 15.3, 7.5)	136.7	C-5, C-2
H-5	5.37 (dd, 10.9, 10.2)	132.7	C-3	H-5	3.55 (m)	3.64 (m)	41.9	C-4, C-6
H-6	4.96 (dd, 10.2, 9.2)	68.9	C-4	H-6	2.02 (m, 2H)	1.95 (m, 2H)	35.8	
H-7a	3.02 (dd, 16.0, 9.2)	41.6	C-6, C-8	H-7	2.51 (m, 2H)	2.44 (m, 2H)	32.7	C-6, C-8, C-13, C-9
H-7b	2.88 (d, 16.0)		C-6, C-8					
C-8	—	131.6	—	C-8	—	—	134.2	—
C-9	6.93 (s)	134.6	—	H-9	7.00 (d, 8.8)	6.83 (d, 8.3)	129.5	C-7, C-11, C-10
C-10	—	126.4	—	H-10	6.71 (d, 8.8)	6.45 (d, 8.3)	115.2	C-8, C-9
C-11	—	150.4	—	C-11	—	—	153.6	—
H-12	6.64 (d, 7.8)	114.2	C-8, C-13, C-10	H-12	6.71 (d, 8.8)	6.45 (d, 8.3)	115.2	C-8, C-13
H-13	6.96 (d, 7.8)	129.2	C-9	H-13	7.00 (d, 8.8)	6.83 (d, 8.3)	129.5	C-7, C-12, C-11
C-1′	—	131.6	—	C-1′	—	—	153.9	—
H-2′	7.49 (d, 2.0)	139.6	C-3′, C-4′, C-1	H-2′	6.73 (d, 2.4)	6.77 (d, 2.9)	114.3	C-4′, C-3′, C-5
C-3′	—	126.4	—	C-3′	—	—	130.8	—
C-4′	—	150.7	—	C-4′	—	—	147.3	—
H-5′	6.76 (d, 7.0)	115.2	C-3′, C-1′, C-4′	H-5′	6.65[c]	6.30 (d, 8.7)	116.8	C-1′, C-6′
H-6′	6.98 (dd, 7.0, 2.0)	127.9	C-2′	H-6′	6.68[c]	6.52 (dd, 8.7, 2.9)	111.9	C-4′, C-2′
OH–C-4′	n.d.			OH–C-4′	4.47 (br s)	3.84 (br s)	—	C-3′, C-5′
OH–C-11	n.d.			OH–C-11	4.69 (br s)	3.87 (br s)	—	C-12, C-10
OH–C-6	n.d.			OMe–C-1′	3.74 (s)	3.37 (s)	55.7	C-1′

n.d. Not detected.
[a] In $CDCl_3$, *J* in hertz.
[b] In $CDCl_3$–C_6D_6 (70:30), *J* in hertz.
[c] Overlapped.

connectivities as presented above were confirmed by the HMBC experiment data which are included in Table 1.

The metabolites **1** and **2** are probably biosynthetically related. Starting from a common precursor, the biphenyl system may be formed by a radical coupling, whereas the formation of the cymodiene system requires a rearrangement of an α,ω-diphenyl precursor. For comparison we have numbered both structures in a similar way.

The cytotoxicities of cymodienol (**1**) and cymodiene (**2**) were assayed against NSCL-N6 and A549 lung cancer cell lines. Metabolite **1** showed strong cytotoxicity: IC_{50} = 84.0 and 114.6 μM against NSCL-N6 and A549 cells,[13,14] whereas metabolite **2** showed only moderate levels of activity. Growth kinetic studies showed that the antiproliferative effect of metabolite **1** was irreversible since discontinuation of the compound supply after 72 h of treatment did not induce normal growth of the treated cells, suggesting a progressive cell cycle blockade. Previous biological activity evaluations on trihydroxy metabolites of the diarylheptanoid class have revealed moderate levels of antioxidant activity.[15]

Acknowledgement

The study was partially supported by the 01ED146 PENED program of the Greek Secretariat for Research and Development.

References and notes

1. Agostini, S.; Pergent, G.; Marchand, B. *Aquat. Bot.* **2003**, *76*, 185–193.
2. Sfriso, A.; Ghetti, P. F. *Aquat. Bot.* **1998**, *61*, 207–223.
3. Sica, D.; Piccialli, V.; Massulo, A. *Phytochemistry* **1984**, *23*, 2609–2611.
4. Cariello, L.; Zanetti, L.; De Stefano, S. *Comp. Biochem. Physiol.* **1979**, *62B*, 159–161.
5. Iliopoulou, D.; Vagias, C.; Pannecouque, Ch.; De Clercq, E.; Roussis, V. *Tetrahedron* **2002**, *58*, 6749–6755.
6. Iliopoulou, D.; Mihopoulos, N.; Vagias, C.; Papazafiri, P.; Roussis, V. *J. Org. Chem.* **2003**, *68*, 7667–7674.
7. Siamopoulou, P.; Bimplakis, A.; Iliopoulou, D.; Vagias, C.; Cos, P.; Vanden Berghe, D.; Roussis, V. *Phytochemistry* **2004**, *65*, 2025–2030.
8. Spectral data of compound **1**: $[\alpha]_D$ −181 (*c* 0.5, CH_2Cl_2); HREIMS: *m/z* $[M]^+$; 294.1221 (294.1256 calculated for $C_{19}H_{18}O_3$); IR (KBr): ν_{max} 3688, 3598, 2360, 2270, 1711, 1602, 1509, 1362, 1273, 1029 cm^{-1}; UV (CH_2Cl_2) λ_{max} (nm) (log ε) 279.4 (1.75), 300.0 (1.84), 385.6 (1.26). NMR data are shown in Table 1.
9. Fuchino, H.; Satoh, T.; Shimizu, M.; Tanaka, N. *Chem. Pharm. Bull.* **1998**, *46*, 166–168.
10. Morihara, M.; Sakurai, N.; Inque, T.; Kawai, K.; Nagai, M. *Chem. Pharm. Bull.* **1997**, *45*, 820–823.
11. Nomura, M.; Tokoroyama, T.; Kubota, T. *Phytochemistry* **1981**, *20*, 1097–1104.
12. Spectral data of compound **2**: $[\alpha]_D$ −17.0 (*c* 0.1, CH_2Cl_2). FAB-MS: *m/z* $[M]^+$; 310.1569 (310.1555 calculated for $C_{20}H_{22}O_3$); IR (KBr): ν_{max} 3804, 3235, 2384, 2277, 1618, 1453, 1329, 1162, 670 cm^{-1}; UV (CH_2Cl_2) λ_{max} (nm) (log ε) 244.8 (2.92), 260.0 (2.16), 286.2 (2.73). NMR data are shown in Table 1.
13. Roussakis, C.; Gratas, C.; Audouin, A. F.; Boterff, J. L.; Dabouis, C.; Andre, M. J.; Moyon, E.; Vo, N. H.; Pradal, G.; Verbist, J. F. *Anticancer Res.* **1991**, *11*, 2239–2244.
14. Giard, D. J.; Aaronson, S. A.; Todaro, G. J.; Arnstein, P.; Kersey, J. H.; Dosik, H.; Parks, W. P. *J. Natl. Cancer Inst.* **1973**, *51*(5), 1417–1423.
15. Lee, J. S.; Kim, H. J.; Park, H.; Lee, Y. S. *J. Nat. Prod.* **2002**, *65*, 1367–1370.