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Prevezols A and B: new brominated diterpenes from the red alga *Laurencia obtusa*

Nikos Mihopoulos,[a,b] Constantinos Vagias,[a] Emmanuel Mikros,[c] Michael Scoullos[b] and Vassilios Roussis[a,*]

[a]*School of Pharmacy, Department of Pharmacognosy, University of Athens, Panepistimioupolis Zografou, Athens 157 71, Greece*
[b]*Department of Chemistry, Division III, Laboratory of Environmental Chemistry and Chemical Oceanography, University of Athens, Panepistimioupolis Zografou, Athens 157 71, Greece*
[c]*School of Pharmacy, Department of Pharmaceutical Chemistry, University of Athens, Panepistimioupolis Zografou, Athens 157 71, Greece*

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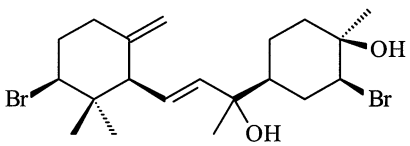
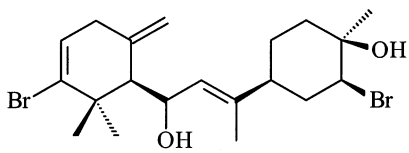
Abstract—Two novel brominated diterpenes, prevezols A and B, have been isolated from the organic extract of the red alga *Laurencia obtusa*, collected from the coastal rocks of Preveza in the Ionean Sea, Greece. The structures of the new natural products, as well as their relative stereochemistry, were established by means of spectral data analysis, including 2D NMR experiments along with molecular calculations.

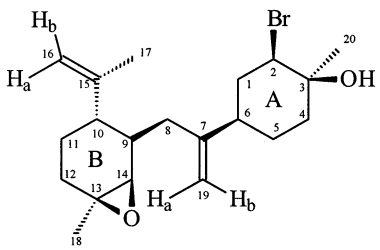
Red algae of the genus *Laurencia* have provided a large number of unique secondary metabolites[1–3] and seem to be an endless source of new chemical constituents. In this study we describe the isolation[4] and structure eluci-



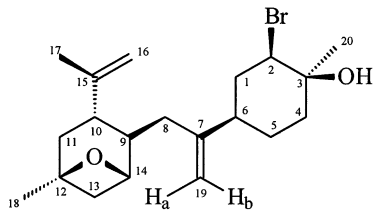
Obtusadiol



Rogioldiol A



Prevezol A (1)



Prevezol B (2)

Figure 1. Structures of the brominated diterpenes obtusadiol, rogioldiol A, prevezol A (**1**) and prevezol B (**2**).

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* Corresponding author. Tel./fax: +301-7274592; e-mail: roussis@pharm.uoa.gr

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dation of two novel brominated diterpenes, prevezols A and B (Fig. 1), which were obtained from the non polar fractions of the organic extract of *Laurencia obtusa*, and showed significant cytotoxicity on the initial *Artemia salina* screening assays ($LC_{50}=6.35$ ppm).

Both ^{13}C NMR data and EI-HRMS measurements of prevezol A (**1**) supported the molecular formula $C_{20}H_{31}BrO_2$.[5] The EI mass spectrum revealed molecular ion $[M]^+$ isotopic peaks at m/z 382 and 384. The isotopic pattern (1:1) as well as the mass fragments indicated the presence of one bromine atom. The IR spectrum of **1** displayed absorptions for a tertiary hydroxyl group (3430 cm^{-1}, broad), and *exo*-methylene groups (3080, 1650, 880 cm^{-1}). The ^{13}C NMR spectrum along with the DEPT experiments showed the presence of four quaternary, five methine, eight methylene and three methyl carbon atoms. From these carbons, three were bonded to oxygens, resonating at δ 70.3 (s), 62.2 (d) 59.2 (s) and one was brominated (δ 65.8 ppm, d). With an unsaturation degree of 5, the structure was suggested to contain two double bonds and three rings. The two double bonds are both exocyclic methylenes and their presence was evident from the methylene peaks at δ 111.6 and 110.1 in the ^{13}C NMR spectrum. Furthermore, the ^1H NMR spectrum showed the appropriate signals at δ 4.69, 4.78, 4.84 and 4.90 (each 1H, br s), the presence of two deshielded methyl groups at δ 1.27 and 1.31 (each 3H, br s) on oxygenated quaternary carbons and one vinyl methyl group at δ 1.63 (3H, br s) (Table 1). In the region 2.3–1.3 ppm the overlap of the resonances is complex and the chemical shifts could only be determined with the help of 2D spectra, thus the multiplicity of these signals was not defined.

Comparison of spectral data of **1** with literature values showed that the structure did not belong to any of the common series of *Laurencia* diterpenoids, such as the labdane-type,[6] or the irieols.[7] Nevertheless, CMR data indicated that part of the molecule was related to the unique brominated *Laurencia* diterpenes obtusadiol[8] and rogioldiol A[9] (Fig. 1). Specifically, the spectral data of the brominated six-membered ring were in good agreement with published values. Strong correlations in the HMBC spectrum between C-6 and C-8 with the methylene protons H-19a and H-19b confirmed the position of one of the terminal methylene groups. The carbon connectivities in the open chain were traced through 2D experiments. The hetero- and homonuclear correlations of H-9 and H-10 with the adjacent carbons established the position of the isopropylidene group on the ring. The low field resonances of the oxygenated carbons supported the presence of an epoxide moiety. The ether bridge was placed on carbons C-13 and C-14 because of the strong correlations of C-9 and C-10 to H-14.

The stereochemical configuration of the asymmetric centers is proposed on the basis of NOE enhancements (Table 1), coupling constants and molecular calculations. The above data indicates that the brominated ring A has the same relative stereochemistry as in the literature.[8,9] Concerning ring B, eight structures were constructed, using the Macromodel[10a] software, taking into account all possible combinations of the configuration of C-9, C-10, C-13 and C-14. The conformational preferences for ring B were explored for all eight structures and the resulting dihedral angle H-9/C-9/C-14/H-14 was used to calculate the theoretical $^3J_{9-14}$ coupling constant on the basis of the appropriate Karplus equation.[11] These data showed that when C-9, C-10, C-13 and C-14 have the configuration *R*,*S*,*S* and *R*, respectively, the theoretical value $^3J_{9-14}=1.3$ Hz is in close agreement with the experimental value 1.4 Hz. However, the enantiomeric combination (of ring B) cannot be excluded as the difference in the corresponding calculated coupling constant $^3J_{9-14}=1.7$ Hz is close to the limit of the coupling constant measurement. A Monte Carlo[10b,c] conformational search was performed for this structure and a number of conformers resulted. The lowest energy conformation is in good agreement with the observed NOEs and is hence, the proposed structure for metabolite **1** (Fig. 1).

Prevezol B (**2**) was purified by HPLC and was isolated as a colorless oil.[12] Both ^{13}C NMR data and EI-HRMS measurements supported the molecular formula $C_{20}H_{31}BrO_2$. Comparison of spectral data of **2** with those of **1** indicated that the structures of the two molecules were very similar. The NMR data of **2** showed resonances corresponding to the brominated ring A, suggesting that this moiety also exists in prevezol B. Concerning ring B, the main differences were observed in the chemical shifts of the oxygen bridge carbons. The chemical shifts of 75.9 and 72.9 ppm were not appropriate for an epoxide ring. One end of the ether linkage should be in proximity to C-8 as the ^{13}C at 75.9 ppm showed long-range coupling with H-8 and the corresponding ^1H at 4.02 ppm shows NOE enhancements with H-8. On this basis, the position of the ether bridge was placed at C-14. The most significant difference of this metabolite compared to prevezol A is the Me shift from C-13 to C-12 and subsequent formation of the ether bridge on this C-12 quaternary carbon. The position of isopropylidene on C-10 is supported by the NOE correlations of this group with H-8. The Monte Carlo conformational search suggested the same configuration for the asymmetric centers as is found in prevezol A. The lowest energy conformation is in agreement with the experimental NOE data and the proposed structure.

Acknowledgements

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Table 1. NMR data for prevezol A (**1**) and B (**2**)

Position	Compound **1**				Compound **2**			
	δ ^{1}H	δ ^{13}C	HMBC	NOESY	δ ^{1}H	δ ^{13}C	HMBC	NOESY
1	2.12 (H-1a, m) 2.19 (H-1b, m)	39.4 t		H-2, H-19b	2.14 (2H, m)	39.4 t	H-6	H-19b
2	4.13 (1H, dd, 4.7, 12.0)	65.8 d	H-1a,b, H-4b, H-20	H-1a,b, H-4a	4.13 (1H)[a]	66.0 d	H-1, H-4b, H-20	H-4b, H-6, H-20
3		70.3 s	H-1a,b, H-4b, H-20			70.4 s	H-1, H-4b, H-20	
4	1.46 (H-4a, m)	37.6 t	H-20		1.47 (H-4a, m)	37.6 t	H-5b, H-6, H-20	
	2.00 (H-4b, m)			H-2	2.04 (H-4b, m)			H-2
5	1.58 (H-5a, m)	26.5 t		H-19b	1.41 (H-5a, m)	26.8 t	H-1, H-4a	
	1.73 (H-5b, m)		H-1a,b, H-4b	H-19b	1.59 (H-5b, m)			H-19b
6	1.95 (1H, m)	45.3 d	H-1a,b, H-4b, H-8b, H-19a,b	H-2	1.98 (1H, m)	45.8 d	H-1, H-4b, H-19a	H-2
7		150.0 s	H-8a,b, H-9			149.6 s	H-6, H-8	
8	1.98 (H-8a, brd, 14.0) 2.22 (H-8b, brd, 14.0)	36.0 t	H-10, H-14, H-19a,b	H-19a	2.23 (2H, m)	38.3 t	H-9, H-19b	H-14, H-17
9	1.94 (1H, m)	36.5 d	H-8b, H-10, H-14	H-14	2.02 (1H, m)	43.1 d	H-8, H-14	H-14
10	1.87 (1H, m)	43.0 d	H-11, H-14, H-16a,b, H-17	H-16a	2.07 (1H, m)	52.6 d	H-13, H-16, H-17	H-17
11	1.80 (2H, m)	28.4 t	H-10		1.46 (H-11a, m) 1.58 (H-11b, m)	28.6 t		
12	1.43 (H-12a, m) 1.60 (H-12b, m)	26.4 t	H-10			72.9 s	H-10, H-13b, H-14, H-18	
13		59.2 s	H-18		1.57 (H-13a, m) 1.96 (H-13b, m)	37.7 t	H-18	H-14
14	2.96 (1H, d, 1.4)	62.2 d	H-9, H-18	H-9, H-18, H-19a	4.02 (1H)[a]	75.9 d	H-8, H-13b, H-18	H-8, H-9, H-13a, H-19a
15		147.3 s	H-11, H-17			146.7 s	H-17	
16	4.69 (H-16a, br s)	111.6 t	H-17	H-10	4.75 (2H, br s)	113.1 t	H-17	
	4.78 (H-16b, br s)			H-17				
17	1.63 (3H, br s)	19.8 q	H-11, H-16a,b	H-16b	1.60 (3H, br s)	19.1 q	H-16	H-10
18	1.27 (3H, br s)	24.3 q	H-14	H-14	1.40 (3H, br s)	23.5 q	H-13b, H-14	
19	4.84 (H-19a, br s)	110.1 t	H-6, H-9	H-8a, H-14	4.86 (H-19a, br s)	110.2 t	H-6, H-8	H-14
	4.90 (H-19b, br s)			H-1a, H-5a,b	4.88 (H-19b, br s)			H-5b
20	1.31 (3H, br s)	30.5 q		H-2	1.31 (3H, br s)	30.5 q		H-2

All spectra were recorded on a Bruker DRX 400, in $CDCl_3$. Chemical shifts are expressed in ppm and J values in parenthesis are in Hz.
[a] Both resonances showed a second order pattern at 400 MHz.

References

1. Faulkner, D. J. *Nat. Prod. Rep.* **1997**, *14*, 259.
2. Erickson, K. L. In *Marine Natural Products*; Scheuer, P. J., Ed.; Academic Press: New York, 1983; Vol. 5, pp. 131–257.
3. Norte, M.; Gonzalez, A. G.; Cataldo, F.; Rodriguez, M. L.; Brito, I. *Tetrahedron* **1991**, *47*, 9411.
4. The alga was collected (151.4 g dry wt) from the coast of Preveza in the Ionean Sea during the summer of 1998 and was extracted with CH_2Cl_2/MeOH. Subsequent normal phase VCC and HPLC separations yielded compounds **1** (2.4 mg) and **2** (9.4 mg) as colorless oils (specimens are deposited at the herbarium of the Laboratory of Pharmacognosy).
5. Prevezol A (**1**): $[\alpha]_D^{20}$ −32.50 ($CHCl_3$, *c* 0.16); IR λ_{max} (film) cm^{-1}: 3430 (broad), 3080, 2950, 2935, 2853, 1650, 1450, 1250, 1234, 1160, 1080, 1010, 930, 900, 880; UV λ_{max} (*n*-hexane) nm (log ε): 205 (3.31); EI-HRMS found: 382.1498; $C_{20}H_{31}{}^{79}BrO_2$ requires 382.1501; EIMS [70 eV, *m*/*z* (rel. int.)]: 384, 382 $[M]^+$ (6:6), 369, 367 (8:8), 353 (23), 267 (19), 133 (69), 122 (88), 93 (100), 81 (85), 79 (82).
6. Howard, B. M.; Fenical, W. *Phytochemistry* **1980**, *19*, 2774.
7. Fenical, W.; Howard, B.; Gifkins, K.; Clardy, J. *Tetrahedron Lett.* **1975**, *46*, 3983.
8. Howard, B. M.; Fenical, W. *Tetrahedron Lett.* **1978**, *28*, 2453.
9. Guella, G.; Marchetti, F.; Pietra, F. *Helv. Chim. Acta* **1997**, *80*, 684.
10. (a) Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comp. Chem.* **1990**, *11*, 440; (b) Chang, G.; Guida, W. C.; Still, W. C. *J. Am. Chem. Soc.* **1989**, *111*, 4379; (c) Saunders, M.; Houk, K. N.; Wu, Y.-D.; Still, W. C.; Lipton, M.; Chang, G.; Guida, W. C. *J. Am. Chem. Soc.* **1990**, *112*, 1419.
11. Haasnoot, C. A. G.; De Leeuw, F. A. A. M.; Altona, C. *Tetrahedron* **1980**, *36*, 2783.
12. Prevezol B (**2**): $[\alpha]_D^{20}$ +33.33 ($CHCl_3$, *c* 0.09); IR λ_{max} (film) cm^{-1}: 3437 (broad), 3080, 2948, 2935, 1643, 1633, 1235, 1166, 1111, 1007, 887; UV λ_{max} (*n*-hexane) nm (log ε): 208 (3.60); EI-HRMS found: 382.1507; $C_{20}H_{31}{}^{79}BrO_2$ requires 382.1501; EIMS [70 eV, *m*/*z* (rel. int.)]: 384, 382 $[M]^+$ (2:2), 369, 367 (3:3), 355 (6), 353 (10), 313 (9), 267 (14), 257 (12), 159 (23), 133 (78), 122 (95), 93 (100), 81 (64), 71 (45).