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PHYTOCHEMISTRY

Geranylgeraniol-derived diterpenoids from the brown alga *Bifurcaria bifurcata*

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Abstract

Three novel diterpenes were isolated from the brown alga *Bifurcaria bifurcata* collected off the Atlantic coast from Brittany, and their structures established by spectroscopic methods. Two of them are acyclic diterpenoids derived from 13-ketogeranylgeraniol, while the third is a β,γ-epoxy-γ-butyrolactone derived from (*S*)-hydroxygeranylgeraniol. Some assignments of ^{13}C- and ^{1}H-NMR signals in 13-ketogeranylgeraniol and related diterpenoids were revised to take account of the results obtained in 2D NMR long-range C–H correlation experiments. These results are discussed from a chemotaxonomic point of view.

Keywords: *Bifurcaria bifurcata*; Cystoseiraceae; Brown alga; Acyclic diterpenes; Geranylgeraniol; Epoxylactone; Chemotaxonomy

1. Introduction

As part of a phytochemical study of the Atlantic brown alga *Bifurcaria bifurcata* (Phaeophyceae), (Combaut & Piovetti, 1983; Valls, Banaigs, Francisco, Codomier & Cave, 1986; Semmak, Zerzouf, Valls, Banaigs, Jeanty & Francisco, 1988; Valls, Banaigs, Piovetti, Archavlis & Artaud, 1993a; Valls, Piovetti, Banaigs, Archavlis and Pellegrini, 1995a; Culioli, Mesguiche, Piovetti & Valls, 1999a), we describe the isolation and structure elucidation of three novel diterpenes. Two of them, isolated from a specimen collected at Quiberon (France) were ketoalcohols derived from 13-ketogeranylgeraniol (**1**), a previously reported acyclic diterpene named eleganolone by its discoverers (Francisco, Combaut, Teste & Prost, 1978). These secondary metabolites were present together with previously studied compounds: **1**, **2** (Biard, Verbist, Floch & Letourneux, 1980), **3a** (Amico, Oriente, Piattelli, Ruberto & Tringali, 1981; Culioli, di Guardia, Valls & Piovetti, 1999b) and geranylgeraniol (Culioli et al., 1999a). The third novel metabolite, isolated from another specimen collected at Roscoff (France), was a β,γ-epoxy-γ-butyrolactone derived from (*S*)-13-hydroxygeranylgeraniol (**2**). It was present together with previous diterpenes described in this extract: **2**, **4**, **5** (Valls et al., 1995a), **1** and geranylgeraniol (Culioli et al., 1999a).

2. Results and discussion

The ether extracts of dried *B. bifurcata* collected at Quiberon and Roscoff, respectively, were fractionated by liquid chromatography using silica gel. The fraction of the Quiberon extract eluted with EtOAc-isooctane (2:3) was further purified by HPLC on normal phase

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Table 1
^{1}H-NMR spectral data of compounds **1** and **6** (TMS as int. standard)[a]

	1		**6**	
H	$CDCl_3$ (250 MHz)[b]	$CDCl_3$ (400 MHz)	$CDCl_3$ (400 MHz)	C_6D_6 (400 MHz)
1	4.15 *d* (6.5)	4.13 *d* (6.5)	4.15 *d* (6.5)	4.02 *d* (6.5)
2	5.40 *t* (6.5)	5.39 *t* (6.5)	5.41 *t* (6.5)	5.40 *t* (6.5)
4	2.04 *m*	2.02 *m*	1.98 *m*	2.00 *m*
5	2.04 *m*	2.09 *m*	2.10 *m*	2.12 *m*
6	5.10 *t* (6.5)	5.10 *t* (6.5)	5.11 *t* (6.5)	5.21 *t* (6.5)
8	2.04 *m*	2.02 *m*	1.98 *m*	2.03 *m*
9	2.04 *m*	2.09 *m*	2.10 *m*	2.11 *m*
10	5.21 *t* (6.5)	5.22 *t* (6.5)	5.23 *t* (6.5)	5.21 *t* (6.5)
12	3.03 *s*	3.01 *s*	3.01 *s*	2.81 *s*
14	6.10 *s*	6.10 *s*	2.26 *d* (6.5)	2.01 *d* (6.5)
15	–	–	2.07 *m*	2.11 *m*
16	1.87 *s*	1.86 *s*	0.87 *d* (7.0)	0.82 *d* (7.0)
17	2.12 *s*	2.12 *s*	0.87 *d* (7.0)	0.82 *d* (7.0)
18	1.60 *s*	1.59 *s*	1.60 *s*	1.60 *s*
19	1.60 *s*	1.59 *s*	1.60 *s*	1.54 *s*
20	1.66 *s*	1.66 *s*	1.65 *s*	1.49 *s*

[a] Chemical shifts are δ values, coupling constants (J in parentheses) are given in Hz; assignments were confirmed by decoupling and 2D NMR experiments (COSY ^{1}H–^{1}H, XHCORR and HMBC).
[b] ^{1}H-NMR data of Amico et al. (1987) added for comparison.

silica. From this separation, we obtained the previously reported diterpene **1**, as well as a small amount of geranylgeraniol and a new compound (**6**). The more polar fraction eluted with EtOAc-isooctane (1:1) led to eleganediol (**2**) and two mixtures of ketoalcohols. After acetylation and purification by HPLC, these mixtures revealed the presence of **3b** and of a new compound (**7b**), respectively. For the Roscoff extract, the fraction eluted by liquid chromatography with hexane-Et_2O (1:1) was further purified by HPLC on nor-

Table 2
^{13}C-NMR spectral data of compounds **1** and **6** (TMS as int. standard)[a]

	1			**6**		
C	$CDCl_3$ (62.5 MHz)[b]	$CDCl_3$ (100 MHz)	DEPT	$CDCl_3$ (100 MHz)	C_6D_6 (100 MHz)	DEPT
1	59.4	59.2	CH_2	59.3	59.3	CH_2
2	124.4	123.4	CH	123.3	125.0	CH
3	139.4	139.4	C	139.6	137.9	C
4	39.7	39.4	CH_2	39.5	39.8	CH_2
5	26.4	26.2	CH_2	26.2	26.6	CH_2
6	124.1	124.0	CH	124.0	124.7	CH
7	135.3	135.0	C	135.0	134.9	C
8	39.4	39.3	CH_2	39.2	39.7	CH_2
9	26.9	26.7	CH_2	26.6	26.9	CH_2
10	123.2	129.1	CH	129.4	129.2	CH
11	129.8	129.5	C	129.4	129.6	C
12	55.5	55.3	CH_2	54.4	54.4	CH_2
13	199.9	199.5	C	209.7	207.3	C
14	129.7	122.8	CH	50.5	50.4	CH_2
15	155.8	155.6	C	24.4	24.4	CH
16	27.7	27.7	CH_3	22.5	22.6	CH_3
17	20.7	20.6	CH_3	22.5	22.6	CH_3
18	16.4	16.3	CH_3	16.4	16.5	CH_3
19	16.0	15.9	CH_3	15.9	16.0	CH_3
20	16.0	16.2	CH_3	16.2	16.2	CH_3

[a] Multiplicities were obtained with DEPT sequences.
[b] ^{13}C-NMR data of Amico et al. (1987) added for comparison (assignments of C-2 and C-6, C-10 and C-14 should be exchanged).

Table 3
^{1}H-NMR spectral data of compounds **3a**, **3b** and **7b** (TMS as int. standard)[a]

H	**3a**[b]	**3b**		7b	
	$CDCl_3$ (270 MHz)	$CDCl_3$ (400 MHz)	C_6D_6 (200 MHz)	$CDCl_3$ (400 MHz)	C_6D_6 (400 MHz)
1	4.12 *d* (6.5)	4.58 *d* (6.5)	4.55 *d* (6.5)	4.58 *d* (6.5)	4.59 *d* (6.5)
2	5.36 *t* (6.5)	5.35 *t* (6.5)	5.34 *t* (6.5)	5.34 *t* (6.5)	5.39 *t* (6.5)
4	2.29 *t* (7.0)	2.34 *t* (7.0)	2.30 *m*	2.05 *m*	1.91 *t* (8.0)
5	2.84 *t* (7.0)	2.81 *t* (7.0)	2.50 *m*	2.10 *m*	2.00 *m*
6	–	–	–	5.12 *t* (7.5)	5.06 *t* (8.0)
8	2.24 *m*	2.31 *t* (7.0)	2.22 *m*	2.29 *t* (7.5)	2.35 *t* (7.5)
9	2.15 *m*	2.18 *m*	2.11 *m*	2.82 *t* (7.5)	2.62 *t* (7.5)
10	5.18 *t* (6.5)	5.24 *t* (6.5)	5.19 *t* (6.5)	–	–
12	3.01 *s*	3.04 *s*	2.95 *s*	3.40 *s*	3.27 *s*
14	6.30 *s*	6.09 *s*	5.97 *s*	6.12 *s*	5.88 *s*
16	1.88 *s*	1.88 *s*	1.61 *s*	1.89 *s*	1.43 *s*
17	2.12 *s*	2.13 *s*	2.11 *s*	2.12 *s*	2.04 *s*
18	1.59 *s*	1.60 *s*	1.47 *s*	5.84 *s*	5.39 *s*
				6.18 *s*	5.70 *s*
19	5.70 *s*	5.76 *s*	5.38 *s*	1.62 *s*	1.43 *s*
	5.96 *s*	6.03 *s*	5.58 *s*		
20	1.67 *s*	1.71 *s*	1.53 *s*	1.70 *s*	1.49 *s*
22	–	2.06 *s*	1.74 *s*	2.07 *s*	1.70 *s*

[a] Chemical shifts are δ values, coupling constants (J in parentheses) are given in Hz; assignments were confirmed by decoupling and 2D NMR experiments (COSY ^{1}H–^{1}H, XHCORR and HMBC).
[b] ^{1}H-NMR data of Amico et al. (1981) added for comparison.

mal phase silica [eluant AcOEt-isooctane (2:3)]. From this separation we obtained pure geranylgeraniol together with **1**, **2**, **4**, **5** and a new compound (**8**).

Diterpene **6**, $C_{20}H_{34}O_2$ (HRMS), was an optically inactive oil, the identity of which was readily established by comparison of its spectral properties with those of eleganolone (**1**) (Francisco et al., 1978; Amico, Neri, Piattelli & Ruberto, 1987). Its acyclic nature was established by the presence of three olefinic protons and three vinyl methyls in the ^{1}H-NMR spectrum (Table 1), and of a saturated carbonyl function in the ^{13}C-NMR spectrum (Table 2), a combination which requires four unsaturations. The all E configuration of the double bonds of the isoprenoid chain was verified with the position of the C-18, C-19 and C-20 methyl signals observed above δ20 in the ^{13}C-NMR spectrum (Couperus, Clague & van Dongen, 1976; Coates, Ley & Cavender, 1978). Its IR spectrum contained hydroxyl ($\nu_{OH} = 3412$ cm^{-1} and $\nu_{CO} = 1010$ cm^{-1}) and carbonyl ($\nu_{C=O} = 1710$ cm^{-1}) absorption bands. As in eleganolone, the presence of an allylic primary alcohol function at the end of the isoprenoid chain was confirmed with the ^{1}H-NMR signals at δ4.15 (2H, *d*, J = 6.5 Hz) and δ5.41 (1H, *t*, J = 6.5 Hz) and with the ^{13}C-NMR signal of the methylene carbon at δ59.3 (Tables 1 and 2).

The main differences from compound **1** were: (i) a base peak at m/z 85 in its EI-mass spectrum followed by m/z 57 instead of m/z 83 and 55, respectively, (ii) a saturated carbonyl absorption at 1710 cm^{-1} in its IR spectrum, (iii) the absence of the olefinic proton atδ6.10 (1H, *s*, H-14) and the presence of six magnetic equivalent protons at δ0.87 (6H, *d*, J = 7.0 Hz, H-16 and H-17) in its ^{1}H-NMR spectrum, (iv) the signal of a saturated carbonyl carbon at δ209.7 (C-13) in its ^{13}C-NMR spectrum instead of an α,β unsaturated one, and those of a methine carbon at δ24.4 (C-15) and a methylene carbon at δ50.5 (C-14) instead of two olefinic carbons at δ155.8 and 129.7, respectively. These data showed that the carbonyl function was linked on one side to an isopropyl group and suggested that **6** was the non-conjugated ketone derived from **1**. The assignment of carbon and proton signals (Tables 1 and 2, respectively) was confirmed by means of homonuclear (COSY) and heteronuclear (XHCORR and HMBC) 2D NMR experiments, in particular regarding the location of methyl and quaternary carbons.

The comparison of the ^{13}C-NMR data of compounds **1** and **6**, including the correlations observed in the ^{1}H–^{13}C long range by inverse detection (HMBC) for **6**, showed that the assignments of C-2 and C-6, C-10 and C-14 in **1** should be exchanged in data given by Amico et al. (1987). This fact led us to revise the location of C in **1**, with the same 2D NMR experiments as for **6**. The revised assignments of NMR signals, in $CDCl_3$, are reported in Tables 1 and 2.

The second novel compound (**7b**) had the molecular formula $C_{22}H_{32}O_4$ (HRMS). It contained the acetate function of a primary hydroxyl group (ν_{max} 1738 cm^{-1}; m/z 300 [M$^+$-HOAc]; δ4.58, *d*, J = 6.5 Hz; –

Table 4
^{13}C-NMR spectral data of compounds **3a**, **3b** and **7b** (TMS as int. standard)[a]

C	**3a**[b]		**3b**			**7b**		
	$CDCl_3$ (20.1 MHz)	DEPT	$CDCl_3$ (100 MHz)	C_6D_6 (50 MHz)	DEPT	CDCl3 (100 MHz)	C6D6 (100 MHz)	DEPT
1	59.5	CH_2	61.1	61.0	CH_2	61.4	61.2	CH_2
2	124.3	CH	118.6	119.6	CH	118.3	119.4	CH
3	138.7	C	141.0	140.8	C	142.1	141.5	C
4	34.1	CH_2	33.7	34.0	CH_2	39.4	39.6	CH_2
5	36.3	CH_2	36.7	36.0	CH_2	26.1	26.4	CH2
6	201.6	C	200.9	199.8	C	124.2	124.3	CH
7	148.8	C	148.0	148.6	C	134.3	134.7	C
8	31.0	CH_2	30.7	31.3	CH_2	34.0	34.3	CH_2
9	27.2	CH_2	26.9	27.2	CH_2	36.1	36.3	CH_2
10	123.3	CH	128.2	128.5	CH	200.7	199.8	C
11	130.8	C	130.4	131.0	C	143.3	144.1	C
12	55.4	CH_2	55.2	55.5	CH_2	46.4	46.7	CH_2
13	199.6	C	199.2	197.5	C	197.1	196.0	C
14	128.6	CH	122.7	123.3	CH	123.3	123.7	CH
15	155.8	C	155.7	154.3	C	156.4	154.9	C
16	20.8	CH_3	27.6	27.4	CH_3	27.7	27.2	CH_3
17	27.7	CH_3	20.6	20.5	CH_3	20.8	20.6	CH_3
18	16.5	CH_3	16.5	16.5	CH_3	126.9	125.7	CH_2
19	124.3	CH_2	124.2	123.5	CH_2	16.1	16.1	CH_3
20	16.5	CH_3	16.3	16.4	CH_3	16.5	16.3	CH_3
21	–	–	171.0	170.1	C	171.2	170.2	C
22	–	–	20.9	20.4	CH_3	21.1	20.6	CH_3

[a] Multiplicities were obtained with DEPT sequences.
[b] ^{13}C-NMR data of Amico et al. (1981) added for comparison (assignments of C-10 and C-14, C-16 and C-17 should be exchanged).

CH_2OAC) and two conjugated carbonyls (ν_{max} 1680 cm^{-1}; δ197.1 and 200.7 ppm), one of them in a –COCH=C(Me)$_2$ unit (*m*/*z* 83 followed by *m*/*z* 55).

Inspection of the ^{1}H- and ^{13}C-NMR data (Table 3 and 4) in comparison with those of the congeners previously described (**1**, **3a** and **3b**) suggested the structure

Table 5
^{1}H-NMR spectral data of compounds **2** and **8** (TMS as int. standard)[a]

	2[b]		**8**	
H	$CDCl_3$ (360 MHz)	C_6D_6 (200 MHz)	$CDCl_3$ (400 MHz)	C_6D_6 (400 MHz)
1	4.10 *d* (6.7)	4.11 *d* (6.6)	–	–
2	5.42 *t* (6.7)	5.50 *t* (6.6)	2.64 *d* (19)	1.87 *d* (19)
			2.75 *d* (19)	2.24 *d* (19)
4	2.00 *m*	2.04 *m*	1.75–1.90 *m*	1.18–1.33 *m*
5	2.08 *m*	2.12 *m*	2.09 *m*	1.77 *m*
6	5.12 *t* (6.7)	5.22 *t* (6.7)	5.01 *t* (6.8)	4.93 *m*
8	2.00 *m*	2.04 *m*	2.00 *m*	1.97 *m*
9	2.08 *m*	2.18 *m*	2.09 *m*	2.09 *m*
10	5.18 *t* (6.7)	5.25 *t* (6.7)	5.12 *t* (6.8)	5.21 *t* (6.8)
12	2.09 *m*	2.21–2.29 *m*	2.07 *m*	2.21 *m*
13	4.37 *ddd* (8.2, 8.2, 5.3)	4.48 *ddd* (8.2, 8.2, 5.1)	4.33 *ddd* (8.3, 8.3, 5.2)	4.46 *ddd* (8.3, 8.3, 5.5)
14	5.16 *d* (8.2)	5.32 *d* (8.3)	5.07 *d* (8.3)	5.31 *d* (8.3)
16	1.69 *s*	1.63 *s*	1.65 *s*	1.61 *s*
17	1.66 *s*	1.58 *s*	1.62 *s*	1.56 *s*
18	1.62 *s*	1.60 *s*	1.59 *s*	1.59 *s*
19	1.57 *s*	1.56 *s*	1.55 *s*	1.43 *s*
20	1.64 *s*	1.54 *s*	5.36 *s*	4.69 *s*

[a] Chemical shifts are δ values, coupling constants (*J* in parentheses) are given in Hz; assignments were confirmed by decoupling and 2D NMR experiments (COSY ^{1}H–^{1}H, XHCORR and HMBC).
[b] ^{1}H-NMR data of Valls et al. (1995a) added for comparison.

Table 6
^{13}C-NMR spectral data of compounds **2** and **8** (TMS as int. standard)[a]

	2[b]			**8**		
C	$CDCl_3$ (90 MHz)	C_6D_6 (50 MHz)	DEPT	$CDCl_3$ (100 MHz)	C_6D_6 (100 MHz)	DEPT
1	59.3	59.3	CH_2	173.5	173.0	C
2	124.3	125.3	CH	36.1	35.7	CH_2
3	139.4	137.6	C	62.3	61.8	C
4	39.5	39.9	CH_2	29.5	29.3	CH_2
5	25.8	26.7	CH_2	23.1	23.1	CH_2
6	123.6	124.9	CH	122.5	123.4	CH
7	134.8	134.9	C	136.9	136.4	C
8	39.4	39.8	CH_2	39.4	39.7	CH_2
9	26.2	26.5	CH_2	26.2	26.4	CH_2
10	127.4	128.2	CH	127.5	128.6	CH
11	131.6	132.1	C	131.9	132.5	C
12	48.2	48.7	CH_2	48.2	48.7	CH_2
13	65.6	66.5	CH	65.5	66.2	CH
14	128.5	129.1	CH	129.2	129.1	CH
15	135.0	133.5	C	135.0	133.7	C
16	26.4	25.8	CH_3	25.8	25.7	CH_3
17	18.2	18.2	CH_3	18.2	18.1	CH_3
18	16.3	16.4	CH_3	16.1	16.3	CH_3
19	15.9	15.9	CH_3	15.9	15.7	CH_3
20	16.2	16.2	CH_3	82.9	82.6	CH

[a] Multiplicities were obtained with DEPT sequences.
[b] ^{13}C-NMR data of Valls et al. (1995a) added for comparison (assignments of C-2 and C-6 should be exchanged).

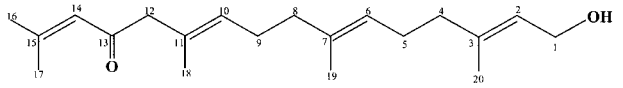
7b for this new compound and **7a** for the metabolite really present in the alga. This structure was confirmed by means of homonuclear (COSY) and heteronuclear (XHCORR and HMBC) 2D NMR experiments. In particular, the location of the ketone at C-10 was precisely determined with the long-range ^2J, $^3J_{C-H}$ chemical shift correlations between C-10/H-12 and C-10/H-9 as well as the location of the olefinic methylene at C-11 with the C-12/H-18 and C-10/H-18 connectivities.

As for eleganolone (**1**), the NMR study of compounds **7b** and **3b** led us to revise the assignment of NMR signals given by Amico et al. (1981) for **3a**. In particular, the ^{13}C-NMR signals of C-10 and C-14 should be exchanged, as well as those of C-16 and C-17 (Table 4).

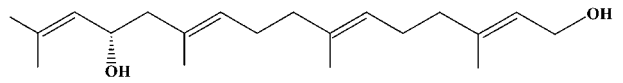
The third novel compound (**8**) had the molecular formula $C_{20}H_{30}O_4$ (HRMS). It is an optically active oil which showed hydroxyl absorption ($\nu_{OH} = 3400$ cm^{-1}) in its IR spectrum, and a base peak m/z 85 (100%) in its EI-mass spectrum, as in the case of **2** and **4**. The ^{1}H- and ^{13}C-NMR data of its acyclic moiety were similar to those of **2** and **4** (Tables 5 and 6), as well as the configuration at the C-13 [Horeau determination (Horeau & Nouaille, 1971)] and at the isoprenoid double bonds (C-18 and C-19 methyls signals above δ20). The presence of a β-substituted-β,γ-epoxy-γ-butyrolactone group included in the first isoprenoid unit was revealed by: (i) the IR absorptions at 1800 cm^{-1} ($\nu_{C=O}$) and 1260 cm^{-1} (ν_{CO}); (ii) the ^{1}H-NMR signals in $CDCl_3$ atδ2.64 (*d*, J = 19 Hz) and δ 2.75 (*d*, J = 19 Hz) corresponding to an AB system obtained from the C-2 methylene, followed by the signal at δ5.36 (*s*) for H-20; these signals were strongly shifted upfield in C_6D_6, at δ1.87, 2.24 and 4.69 (Table 5); (iii) the ^{13}C-NMR assignments in $CDCl_3$ of two oxygenated sp^3 carbons, one quaternary at δ62.3 (C-3) and the other tertiary at δ82.9 (C-20), of a non-oxygenated methylene at δ36.1 (C-2) and of a quaternary carbon at δ173.5 (C-1, lactone C=O), (Table 6). These assignments were confirmed by homo- and heteronuclear 2D NMR experiments (^{1}H–^{1}H COSY, XHCORR and HMBC). The main long-range ^{2}J, $^3J_{C-H}$ chemical shift correlations were the C-1/H-2, C-1/H-20, C-3/H-2 connectivities, as well as C-3/H-4, C-20/H-2 and C-20/H-4. These last correlations enabled us to specify that both epoxyde and lactone of the β,γ-epoxy-γ-lactone moiety were linked to the acyclic chain by C-3. It must be specified that in this case, the acyclic chain is linked in β from the lactone C=O instead of α in **5**, a previous metabolite isolated from the extract (Valls et al., 1995a). As **5**, the new compound **8** could be an oxidation product of bifurcane (**4**), as has been shown by the autoxidation studies on the marine furanosesterterpene variabilin (Barrow, Blunt & Munro, 1989).

2.1. Chemotaxonomic relationships

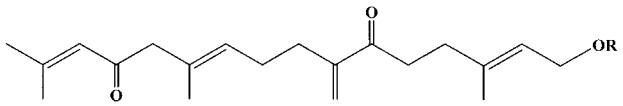
The re-investigation of the lipid extracts from



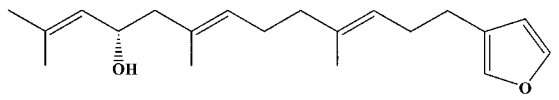
1



2



3a R=H
3b R=Ac



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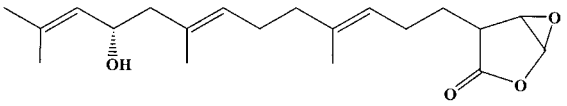
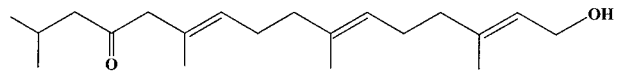
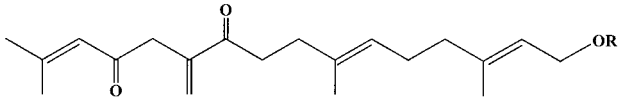


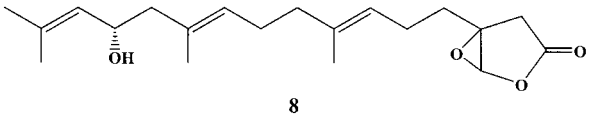
5



6



7a R=H
7b R=Ac



8

Bifurcaria bifurcata completed our previous work on the identification of the minor constituents. The results suggested that: (i) the presence of geranylgeraniol — recently isolated for the first time from the genus *Bifurcaria* (Culioli et al., 1999a) — in the specimen collected at Quiberon, simultaneously with **1**, **2**, **3a** and **7a** showed that this species is closely related to the Mediterranean Cystoseiraceae species: *Cystoseira brachycarpa* (Amico et al., 1981; Amico, Oriente, Piattelli, Ruberto & Tringali, 1980), initially mistakenly called *C. crinita* (Amico, Piattelli, Neri & Ruberto, 1988), and *C. balearica* (Amico et al., 1987; Della Pietà, Bilia, Breschi, Cinelli, Morelli & Scatizzi, 1993; Della Pietà, Breschi, Scatizzi & Cinelli, 1995), which were the only Cystoseira species with linear diterpenes (Valls, Piovetti & Praud, 1993b; Valls & Piovetti, 1995b); (ii) the identification of eleganolone (**1**) as a minor constituent of the ether extract from *B. bifurcata* collected at Roscoff, showed that this compound — not obtained in our previous study of this extract (Valls et al., 1995a) — can be regarded as a chemotaxonomic marker of the species (Culioli et al., 1999a).

3. Experimental

3.1. General

MS: direct inlet, 70 eV; ^{1}H-NMR: 200 and 400 MHz; ^{13}C-NMR: 50 and 100 MHz. Chemical shifts are quoted in ppm (δ) relative to TMS and coupling constants are in Hz. Final purification of all metabolites was achieved by HPLC on silica gel (Lichrosorb Si-60, 5 μm) with RI monitoring.

3.2. Plant material

Bifurcaria bifurcata Ross was collected near Quiberon and Roscoff, Brittany, France in December 1996 and July 1992, respectively. A voucher specimen of each specimen of this species was deposited in the herbarium of Dr. Pellegrini, Laboratoire de Biologie Marine Fondamentale et Appliquée, University of Marseille II, France.

3.3. Extraction and purification

The shade-dried material collected from Quiberon (650 g) was ground and extracted with Et_2O at room temperature. After filtration, the filtrate was evaporated to yield 20.5 g of a crude extract which was subjected to CC on silica gel eluted with a solvent gradient from isooctane to EtOAc. The first new compound (**6**) with **1** and geranylgeraniol was eluted with EtOAc-isooctane (2:3) and subsequently purified by semi-preparative normal phase HPLC (EtOAc-isooctane, 1:1) to give **1** (76 mg), **6** (90 mg) and geranylgeraniol (50 mg). The second new compound (**7a**) with **2** and **3a** was eluted with EtOAc-isooctane (1:1) and subsequently purified by HPLC (EtOAc-isooctane, 1:1) to give **2** (370 mg), and two mixtures (220 and 105 mg) containing **3a** and **7a**, respectively. These mixtures, after acetylation and purification by HPLC (EtOAc-isooctane, 2:3) led to **3b** (140 mg) and **7b** (60 mg).

In the same way, the freeze-dried material collected from Roscoff (500 g) was ground and extracted with Et_2O to yield 12.0 g of a crude extract which was subjected to CC on silica gel eluted with a solvent gradient from hexane to Et_2O. The third new compound (**8**), with **4**, **5**, **1**, **2** and geranylgeraniol was eluted with hexane-Et_2O (1:1) and subsequently purified by HPLC (EtOAc-isooctane 2:3) to give **1** (15 mg), **2** (200 mg), **4** (300 mg), **5** (60 mg), **8** (35 mg) and geranylgeraniol (25 mg).

3.4. Compound **6**

Oil; IR ν_{max}^{film} cm^{-1}: 3412, 2956, 2927, 2871, 1710, 1617, 1445, 1383, 1367, 1010; HRMS: 306.2558$[M]^+$ (calculated for $C_{20}H_{34}O_2$, 306.2559); EIMS (70 eV) *m/z* (rel. int.): 306$[M]^+$(0.4), 288$[M\text{-}H_2O]^+$(0.9), 211(3), 188(6), 135(12), 121(24), 107(12), 93(15), 85(100), 81(11), 68(29), 57(60), 41(19); ^{1}H- and ^{13}C- NMR: Tables 1 and 2.

3.5. Compound **7b**

Oil; IR ν_{max}^{film} cm^{-1}: 3093, 2975, 2930, 2864, 1738, 1680, 1620, 1443, 1380, 1234, 1108, 1024, 953; UV $\lambda_{max}^{CHCl_3}$ nm (ε): 245 (14,000); HRMS: 360.2297$[M]^+$ (calculated for $C_{22}H_{32}O_4$, 360.2300); EIMS (70 eV) *m/z* (rel. int.): 360$[M]^+$(0.1), 300$[M\text{-}HOAc]^+$(0.5), 277(2.9), 217(4.3), 207(3.4), 199(1.5), 151(20.4), 149(6.7), 134(4.6), 123(3.8), 119(3.2), 107(2.7), 95(5.1), 83(100), 77(2.1), 60(1.0), 55(19.3); ^{1}H- and ^{13}C-NMR: Tables 3 and 4.

3.6. Compound **8**

Oil; $[\alpha]_D^{25}$ −0.9° (CH_2Cl_2; *c* 2.8); IR ν_{max}^{film} cm^{-1}: 3400, 2930, 1800, 1450, 1380, 1260, 1150, 1040, 915, 820; HRMS: 316.2044 $[M\text{-}H_2O]^+$ (calculated for $C_{20}H_{28}O_3$, 316.2038); EIMS (70 eV) *m/z* (rel. int.): 316 $[M\text{-}H_2O]^+$(0.4), 207(4), 135(6), 123(5), 109(7), 107(7), 93(12), 85(100), 69(19), 68(27), 67(15), 55(14); ^{1}H- and ^{13}C-NMR: Tables 5 and 6.

3.7. Absolute configuration at C-13 in **8**

The alcohol **8** (15 mg) in dry pyridine (150 μl) was treated with (±)-2-phenyl-butyric anhydride (50 mg) and left overnight at room temperature. H_2O (500 μl) was added and the mixture warmed for 30 min until a homogeneous solution obtained. H_2O (2 ml) and C_6H_6 (3 ml) were added and the mixture titrated with NaOH (0.1 M) until alkaline (phenolphthalein). C_6H_6 (10 ml) was added and the layers separated The C_6H_6 layer was washed with H_2O and the combined aq. phases acidified (pH 1.5) with HCl (10 M) and extracted with C_6H_6 (2 × 10 ml). The C_6H_6 extracts were washed with H_2O (10 ml), dried over $MgSO_4$ and concentrated. The observed rotation was −0.03. As the acid excess was laevorotatory, compound **8** had the 13(S) configuration.

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