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Polar acyclic diterpenoids from *Bifurcaria bifurcata* (Fucales, Phaeophyta)

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Abstract

From the lipophilic extract of the brown alga *Bifurcaria bifurcata* collected off the Atlantic coast of Southern Brittany (Quiberon, France), five polar linear diterpenoids have been isolated. These metabolites have been identified as hydroxylated derivatives of 13-oxo- and 13-hydroxygeranylgeraniol. Their structures were characterized on the basis of chemical and spectral evidence including two-dimensional NMR experiments and mass spectrometric techniques. The absolute configuration of the 13-position has been determined, for the 13-hydroxygeranylgeraniol derivatives, to be *R* by means of a modified Mosher's method and therefore that of 13-hydroxygeranylgeraniol (eleganediol) has been revised. Along with these compounds, three related known geranylgeraniol derivatives were also identified, and these data were used for chemotaxonomical purposes.

Keywords: *Bifurcaria bifurcata*; *Cystoseira brachycarpa*; Cystoseiraceae; Brown alga; Heterokontophyta; Linear diterpenes; 13-Oxo- and (*R*)-13-hydroxygeranylgeraniol derivatives; Chemotaxonomy

1. Introduction

Brown algae have been shown to possess the ability to produce a great variety of secondary metabolites with very different skeleton types and functionalities (Blunt et al., 2004). In conclusion to our search for compounds with chemotaxonomic interest, from the Phaeophyceae *Bifurcaria bifurcata*, we have re-investigated the lipid extract of this alga collected in Southern Brittany (Quiberon, France). Previous chemical investigations have shown that this species was able to biosynthesize a great number of linear diterpenes (Culioli et al., 2004, and refences cited therein). These compounds were geranylgeraniol (**12a**) and geranylgeraniol-derived diterpenoids such as eleganolone (13-oxogeranylgeraniol, **9a**) and eleganediol (13-hydroxygeranylgeraniol, **11**) for the major ones.

The examination of minor lipophilic secondary metabolites of *B. bifurcata* from Quiberon has conducted us to isolate five new geranylgeraniol derivatives (compounds **1**–**5**). These compounds were relatively polar, possessing three oxygenated functions, in comparison to those previously isolated from the same location (Culioli et al., 1999a,b, 2000). Except **1**, all these

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metabolites were obtained as an optically active oil. Three of them (**1**–**3**) were 13-oxogeranylgeraniol-derivated diterpenes and the two others (**4** and **5**) were 13-hydroxygeranylgeraniol derivatives. The absolute configuration at C-13 of compounds **4** and **5** was determined, by a modified Mosher's method, to be *R* and consequently stereochemistry at C-13 was revised for eleganediol ((*R*)-13-hydroxygeranylgeraniol, **11**). In addition to these five new compounds, three known acyclic diterpenes (**6**–**8**), already described from a mediterranean Cystoseiraceae (*Cystoseira brachycarpa* (Amico et al., 1981)), have been characterized and some assignments of their NMR signals have been revised.

In a chemotaxonomic point of view, their occurrence in this crude extract of *B. bifurcata* has contributed to confirm the existence of a close chemical relationship between this atlantic species and varieties of the mediterranean species *C. brachycarpa* (Amico, 1995; Culioli et al., 2000; Valls and Piovetti, 1995).

2. Results and discussion

The chloroform/methanol extracts of dried *B. bifurcata* collected at Quiberon were filtered and fractionated by liquid chromatography using silica gel with 0–100% EtOAc in cyclohexane as eluent. Further individual purifications of fractions from this column have furnished the known compounds **6**–**8**, and the new diterpenes **1**–**5** in order of increasing polarity: **1**–**3** and **6**–**8** were obtained from the fractions eluted by cyclohexane–EtOAc (2:3); while **4** and **5** were purified from fractions eluted with the mixture cyclohexane–EtOAc (1:4). The final purification of all these products was carried out by HPLC on a C_{18} reversed-phase column.

The molecular formula of compound **1** was determined as $C_{20}H_{34}O_3$, by HREIMS ($[M]^+$, *m*/*z* 322.2511). The ^{13}C NMR data for **1** (multiplicities of the carbon signals shown in Table 2 were determined from the DEPT 45°, 90° and 135° spectra) revealed the presence of one carbonyl, one sp^3 quaternary carbon, five methyls, seven methylenes and six olefinic carbons (three =CH– and three quaternaries). The IR spectrum showed significant absorption bands at ν_{max} 3522 cm^{-1} due to hydroxyl groups and, at 1703 and 1679 cm^{-1}, attributable, respectively, to an unconjugated keton carbonyl and double bonds. As deduced from the ^1H and ^{13}C NMR data (Tables 1 and 2), the hydroxyl groups corresponded to a primary alcohol with signals at δ_H 4.15 (2H, *d*, H-1) and δ_C 59.3 (*t*, C-1), and a tertiary alcohol with signal at δ_C 69.6 (*s*, C-15). The occurrence of the ketone was confirmed by the signal at δ_C 212.0 (*s*, C-13) and was further substantiated by a characteristic mass fragment at *m*/*z* 83 corresponding to the subsequent α-keto-β-allylic break (C_{12}–C_{13}) with loss of H_2O. Also noted in the ^1H NMR spectrum, signals of three olefinic protons and three vinyl methyls pointed out the presence of three trisubstituted isoprenoidal

Table 1
^1H NMR spectral data of compounds **1**–**5** ($CDCl_3$, 400 MHz)[a,b]

H	1	2	3	4	5
1	4.15 (*d*, 6.8)	a: 3.69 (*dd*, 11.2, 2.7) b: 3.53 (*dd*, 11.2, 7.3)	4.14 (*d*, 6.8)	4.15 (*d*, 6.8)	4.16 (*d*, 6.8)
2	5.41 (*t*, 6.8)	4.20 (*dd*, 7.3, 2.7)	5.43 (*t*, 6.8)	5.45 (*t*, 6.8)	5.44 (*t*, 6.8)
3	–	–	–	–	–
4	2.04 (*m*)	2.01 (*m*)	2.05–2.11 (*m*)	2.03–2.10 (*m*)	2.74 (*d*, 4.8)
5	2.12 (*m*)	2.17 (*m*)	1.66 (*m*)	1.66 (*m*)	5.57 (*dt*, 15.5, 4.8)
6	5.11 (*t*, 6.8)	5.12 (*t*, 7.0)	4.06 (*t*, 6.4)	4.05 (*t*, 6.3)	5.61 (*d*, 15.5)
7	–	–	–	–	–
8	2.02 (*m*)	2.02 (*m*)	2.06–2.18 (*m*)	2.05–2.15 (*m*)	1.60 (*m*)
9	2.14 (*m*)	2.13 (*m*)	2.21 (*m*)	2.22 (*m*)	2.15 (*m*)
10	5.26 (*t*, 7.0)	5.22 (*t*, 7.0)	5.24 (*t*, 6.5)	5.25 (*t*, 6.7)	5.27 (*t*, 7.0)
11	–	–	–	–	–
12	3.03 (*s*)	3.02 (*s*)	3.02 (*s*)	2.13 (*m*)	2.12 (*m*)
13				4.45 (*ddd*, 8.4, 8.4, 5.4)	4.43 (*ddd*, 8.2, 8.2, 5.3)
14	2.61 (*s*)	6.10 (*s*)	2.27 (*d*, 6.7)	5.15 (*d*, 8.4)	5.15 (*d*, 8.2)
15			2.11 (*m*)	–	–
16	1.23 (*s*)	1.87 (*s*)	0.89 (*d*, 6.5)	1.72 (*s*)	1.72 (*s*)
17	1.23 (*s*)	2.13 (*s*)	0.89 (*d*, 6.5)	1.69 (*s*)	1.69 (*s*)
18	1.62 (*s*)	1.61 (*s*)	1.60 (*s*)	1.68 (*s*)	1.67 (*s*)
19	1.60 (*s*)	1.61 (*s*)	a: 5.05 (*s*) b: 4.87 (*s*)	a: 5.06 (*s*) b: 4.88 (*s*)	1.28 (*s*)
20	1.68 (*s*)	a: 5.14 (*s*) b: 4.97 (*s*)	1.67 (*s*)	1.69 (*s*)	1.65 (*s*)

[a] δ in ppm (TMS as internal standard), coupling constants (*J* in parentheses) are given in Hz.
[b] Assignments were confirmed by decoupling and 2D NMR experiments (COSY ^1H–^1H, ^1H *J*-resolved, HMQC and HMBC).

Table 2
^{13}C NMR spectral data of compounds **1**–**5** ($CDCl_3$, 100 MHz)[a,b]

C	1	2	3	4	5
1	59.3 (*t*)	65.6 (*t*)	59.3 (*t*)	59.3 (*t*)	59.4 (*t*)
2	123.4 (*d*)	75.0 (*d*)	123.6 (*d*)	123.6 (*d*)	124.3 (*d*)
3	139.5 (*s*)	148.2 (*s*)	139.3 (*s*)	139.4 (*s*)	138.3 (*s*)
4	39.5 (*t*)	32.4 (*t*)	35.6 (*t*)	35.6 (*t*)	42.3 (*t*)
5	26.2 (*t*)	26.3 (*t*)	33.3 (*t*)	33.4 (*t*)	125.2 (*d*)
6	124.2 (*d*)	123.8 (*d*)	74.9 (*d*)	74.8 (*d*)	138.9 (*d*)
7	134.8 (*s*)	135.3 (*s*)	151.3 (*s*)	151.4 (*s*)	73.0 (*s*)
8	39.2 (*t*)	39.2 (*t*)	30.8 (*t*)	31.0 (*t*)	42.4 (*t*)
9	26.6 (*t*)	26.6 (*t*)	26.6 (*t*)	26.5 (*t*)	23.1 (*t*)
10	130.2 (*d*)	129.0 (*d*)	129.1 (*d*)	127.4 (*d*)	127.5 (*d*)
11	128.2 (*s*)	129.5 (*s*)	129.6 (*s*)	132.1 (*s*)	131.7 (*s*)
12	55.4 (*t*)	55.3 (*t*)	54.2 (*t*)	48.1 (*t*)	48.1 (*t*)
13	212.0 (*s*)	199.7 (*s*)	209.7 (*s*)	65.7 (*d*)	65.9 (*d*)
14	51.5 (*t*)	122.8 (*d*)	50.8 (*t*)	128.0 (*d*)	128.6 (*d*)
15	69.6 (*s*)	155.8 (*s*)	24.4 (*s*)	135.0 (*s*)	134.6 (*s*)
16	29.2 (*q*)	27.7 (*q*)	22.5 (*q*)	25.8 (*q*)	25.8 (*q*)
17	29.2 (*q*)	20.7 (*q*)	22.5 (*q*)	18.2 (*q*)	18.2 (*q*)
18	16.4 (*q*)	16.4 (*q*)	16.5 (*q*)	16.3 (*q*)	16.4 (*q*)
19	15.9 (*q*)	16.0 (*q*)	110.1 (*q*)	110.1 (*t*)	28.5 (*q*)
20	16.3 (*q*)	110.8 (*t*)	16.3 (*q*)	16.2 (*q*)	16.2 (*q*)

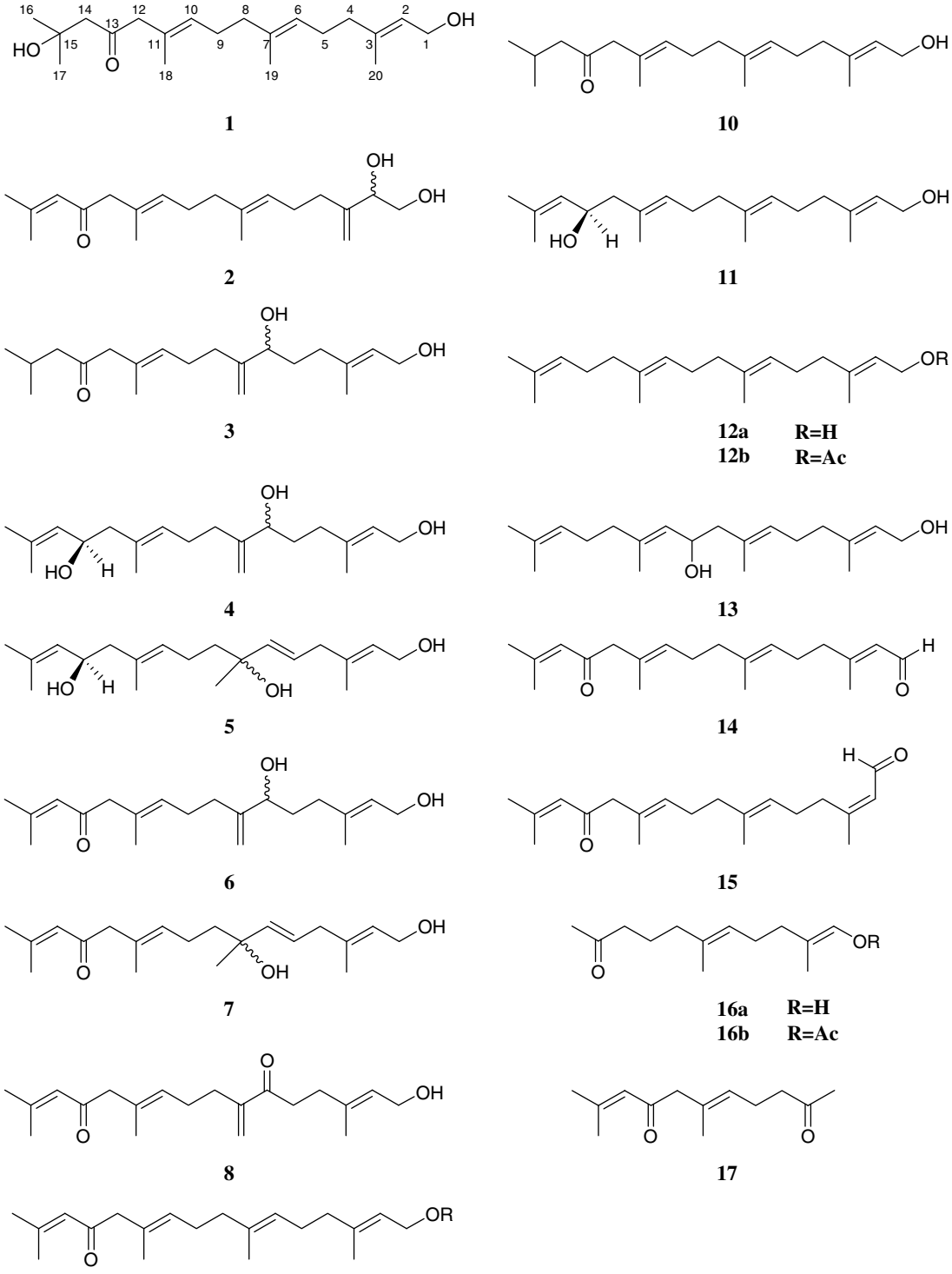
[a] δ in ppm (TMS as internal standard).
[b] Multiplicities were obtained with DEPT sequences. Assignments were made with the aid of HSQC and HMBC experiments.

double bonds. In comparison to eleganolone (**9a**) (Culioli et al., 1999a), this product possessed four isoprenic units minus a double bound according to the count of unsaturations and an extra hydroxyl group according to the molecular formula, the spectroscopic analysis and the relative polarity of the product. Analysis of the NMR data revealed that **1** differed from **9a** by the replacement of the $\Delta^{14,15}$ double bond by an hydroxyl group at C-15. This hypothesis was supported by the presence of (i) a methylene at δ_H 2.61 (2H, *s*, H-14) instead of an olefinic proton, (ii) six magnetically equivalent protons at δ_H 1.23 (6H, *s*, H-16 and H-17) instead of two vinyl methyls, and (iii) two sp^3 carbons at δ_C 69.6 (*s*, C-15) and δ_C 51.5 (*t*, C-14) instead of two olefinic carbons. Finally, the relative stereochemistry of **1** was elucidated on the basis of the NOESY experiment: it showed spatial correlations between H-1/H-20, H-5/H-19 and H-9/H-18, confirming the *E* configuration of the double bonds at C-2, C-6 and C-10, respectively.

The HREIMS mass spectrum of compound **2** gave a molecular peak at *m/z* 320.2353 ($[M]^+$) corresponding to the molecular formula $C_{20}H_{32}O_3$ with two protons less than in compound **1**. The IR spectrum displayed absorption bands for hydroxyl (ν_{max} 3466 cm^{-1}) and conjugated carbonyl (ν_{max} 1687 cm^{-1}) functionalities. This last function was confirmed on the mass spectrum by a base peak at *m/z* 83 (α-keto-β-allylic break at C_{12}–C_{13}) and on NMR data by signals at δ_C 199.7 (*s*, C-13) and δ_H 6.10 (1H, s, H-14), while hydroxyl groups were attributed to a primary and a secondary alcohols with signals at, respectively, δ_C 65.6 (*t*, C-1) and δ_C 75.0 (*d*, C-2). When compared to **1** and **9a**, this compound showed either an extra double bond or an additional hydroxyl group. As compound **2** exhibited similar NMR data to **9a** except for signals belonging to atoms of the first isoprenic unit, it was logical that the extra alcohol function will be found in this part of the molecule. More precisely, the localization of the two hydroxyl groups at C-1 and C-2 was confirmed by NMR on the basis of the non-equivalence of the protons beared by C-1 (signals at δ_H 3.69 (1H, *dd*, H-1a) and δ_H 3.53 (1H, *dd*, H-1b)) and their strong COSY correlation with the signal at δ_H 4.20 (1H, *dd*, H-2). In addition, the unusual position in this isoprenic unit of the $\Delta^{3,20}$ double bond was deduced by NMR regarding the lost of a vinyl methyl in comparison to compound **9a**, the signals at δ_H 5.14 (1H, *s*, H-20a) and δ_H 4.97 (1H, *s*, H-20b) which were correlated on the HSQC spectrum to an olefinic methylene at δ_C 110.8 (*t*, C-20), and the HMBC correlations between H-1/C-2, H-1/C-3, H-2/C-3, H-2/C-1, H-2/C-20, H-20/C-3 and H-20/C-2. Finally, the *E* configuration of the double bonds at C-6 and C-10 was elucidated on the basis of NOESY experiment and all spectroscopic data were consistent with the proposed structure for compound **2**.

As deduced by HREIMS ($[M]^+$, *m/z* 322.2501), compound **3** was determined as an isomer of **1** with a $C_{20}H_{34}O_3$ molecular formula. The IR absorptions at ν_{max} 3401, 1711 and 1620 cm^{-1} indicated the presence of hydroxyl, carbonyl and olefinic groups, respectively. The unconjugated nature of the carbonyl group was directly confirmed by observation of cross-peaks on the HMBC spectrum between signals at δ_C 209.7 (*s*, C-13) and δ_H 2.27 (2H, *d*, H-14), and as for compound **1**, by the mass fragment at *m/z* 83. Characteristic NMR signals of an isopropyl moiety (see Tables 1 and 2) and HMBC correlations between C-13/H-14, C-13/H-15 and C-13/H-12 were in accord with the proposed structure for the last isoprenic unit of compound **3**. As the ^1H NMR spectra showed only two vinyl methyls and because of signals at δ_H 4.06 (1H, *t*, H-6) and δ_C 74.9 (*d*, C-6) due to a secondary alcohol correlated by HMBC to an olefinic methylene (δ_C 110.1 (*t*, C-19), δ_H 5.05 (1H, *s*, H-19a) and δ_H 4.87 (1H, *s*, H-19b)), the chemical structure of the second isoprenic unit of **3** has been defined as it showed on its chemical formula. A combination of 2D NMR experiments allowed us to assign all the NMR chemical shifts (Tables 1 and 2) and to attribute a *E* configuration for the $\Delta^{2,3}$ and $\Delta^{10,11}$ double bonds. These NMR data have been also confirmed by comparison with those of structurally similar molecules: compound **6**, a known diterpenoid described in this study for the first time from *B. bifurcata*, and compound **10**, a linear diterpene already isolated from this species (Culioli et al., 1999a).

Compound **4**, $C_{20}H_{34}O_3$ (HREIMS; $[M]^+$, *m/z* 322.2510), isolated as an optically active yellow oil, was an isomer of **1** and **3** from which it was closely



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structurally related. In fact, while **1** and **3** were derived from 13-oxogeranylgeraniol (**9a**), compound **4** was identified as a 13-hydroxygeranylgeraniol (**11**) derivative through NMR signals at δ_C 65.7 (*d*, C-13) and δ_H 4.45 (1H, *ddd*, H-13) of a secondary alcohol localized at C-13, and the lack of signals due to a carbonyl moiety around 1700 cm^{-1} and 200 ppm on its respective IR and ^{13}C NMR spectra. Complete analysis of NMR data

suggested the occurrence of a secondary alcohol function (δ_H 4.05 (1H, *t*, H-6) and δ_C 74.8 (*d*, C-6)) correlated on the HMBC spectrum to an olefinic methylene (δ_C 110.1 (*t*, C-19), δ_H 5.06 (1H, *s*, H-19a) and δ_H 4.88 (1H, *s*, H-19b)) by cross-peaks between H-6/C-19, H-6/C-7 and H-19a,b/C-6. After comparing the ^{13}C NMR spectra of **11**, **3** and **6** with the one of **4**, it was possible to assign unambiguously to compound **4** a chemical structure with the same last isoprenic unit as **11** and the same second isoprenic unit as **3** and **6**.

In the mass spectrum of the fifth new metabolite **5**, the molecular ion at *m/z* 322.2505 (HREIMS) was in accord with molecular formula $C_{20}H_{34}O_3$ while its IR spectra displayed a strong hydroxyl absorption at ν_{max} 3394 cm^{-1}. This absorption was consistent with ^{13}C NMR signals at δ_C 59.4 (*t*, C-1), δ_C 73.0 (*s*, C-7) and δ_C 65.9 (*d*, C-13), correlated by HSQC with ^1H NMR signals at δ_H 4.16 (2H, *d*, H-1) and δ_H 4.43 (1H, *ddd*, H-13) which corresponded, respectively, to one primary, one tertiary and one secondary alcohol. NMR data of **5** were similar from those of eleganediol (**11**) except signals of atoms belonging to the second isoprenic unit. The chemical structure of this isoprenic part has been deduced by (i) the disubstituted nature of the $\Delta^{5,6}$ double bond revealed by the DEPT multiplicity of signals at δ_C 125.2 (*d*, C-5) and δ_C 138.9 (*d*, C-6), (ii) the position of the hydroxyl group at C-7 and the $\Delta^{5,6}$ double bond which were determined by long range $^2J_{C-H}$ and $^3J_{C-H}$ chemical shift correlations between, respectively, C-7/H-19, C-7/H-6 and C-7/H-5, C-6/H-19, C-6/H-4, C-3/H-5. Thus, the observation on the ^1H NMR spectra of an ABX_2 system at δ_H 5.61 (1H, *d*, H-6), δ_H 5.57 (1H, *dt*, H-5) and δ_H 2.74 (2H, *d*, H-4) was in accord with the proposed structure. For this compound, the configuration of the double bonds at C-2 and C-5 were confirmed as being *E* through spatial NOE correlations, and for $\Delta^{5,6}$ these data were in agreement with the observed large coupling constant between H-5 and H-6 ($^3J_{5,6} = 15.5$ Hz).

Concerning the determination of the absolute configuration of asymmetric carbons in all these compounds, the application of modified Mosher's method (Dale and Mosher, 1973; Ohtani et al., 1991a,b) has been considered. It was not, however, possible to establish the configuration at C-2 in **2**, C-6 in **3**, **4** and **6** or C-7 in **5** and **7** because of the very low quantity purified of each product and (i) for **3**, **4** and **6**, the methylenic environment of the asymmetric centre which made difficult the attribution of the corresponding protons in the ^1H NMR spectra of the *R*- and *S*-MTPA esters, (ii) for **2**, the proximity of an other hydroxyl group at the extremity of the molecule, (iii) for **5** and **7**, the quaternary nature of the asymmetric carbon which prevent the esterification. On the other hand, the absolute configuration of C-13 in **4**, **5** and **11** was readily determined on the basis of the ^1H NMR data of the *R*- and *S*-MTPA esters of these products. From the MTPA determination rule (Ohtani et al., 1991a,b), the positive and negative $\Delta\delta$ value ($\Delta\delta = \delta_{S\text{-MTPA ester}} - \delta_{R\text{-MTPA ester}}$) observed for the signals of protons which were located on the left and right side of the MTPA plane, respectively, showed clearly that the absolute configurations at C-13 was *R* (Fig. 1). As in the case of 12-hydroxygeranylgeraniol derivatives described from *B. bifurcata* (Culioli et al., 2004), these results were in contradiction with the 13*S*-configuration found for eleganediol (**11**) with the Horeau's method (Culioli et al., 1999b; Valls et al., 1995).

In a chemotaxonomic point of view, this work allowed us to confirm the very close chemical relationship between *B. bifurcata* and *C. brachycarpa* (Amico, 1995; Culioli et al., 2000; Valls and Piovetti, 1995). This last species, already chemically studied in the past, was cited under various erroneous names such as *C. crinita*, *C. balearica*, *C. brachycarpa* var. *balearica* (Amico et al., 1980, 1981, 1987, 1988; Della Pieta et al., 1993). Now, this species is only recognized under its two varieties which are distributed along infralittoral zone: *C. brachycarpa* var. *brachycarpa* in the upper part and *C. brachycarpa* var. *claudiae* in the lower part (Cormaci et al., 1992). In this work, compounds **6**–**8** have been described for the first time from the lipidic extract of *B. bifurcata*, but these metabolites had been previously isolated from *C. brachycarpa* (initially mistakenly called *C. crinita* (Amico et al., 1981)). The complete NMR study of these compounds (Tables 3 and 4) led us to revise the assignment of some NMR signals previously made by Amico et al. (1981).

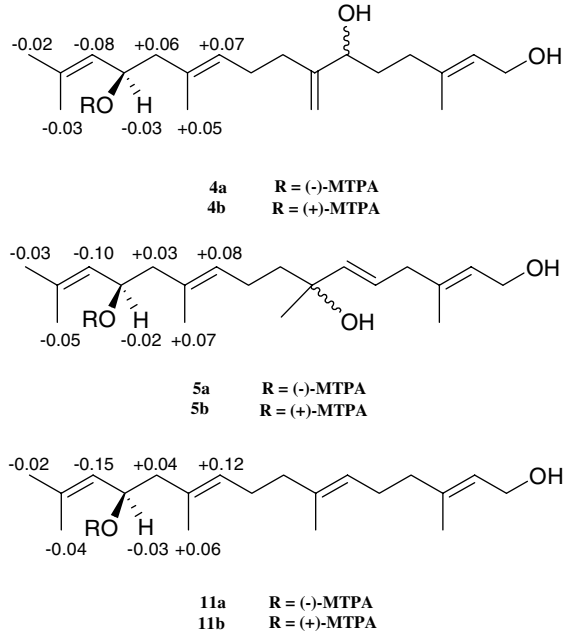

Fig. 1. $\Delta\delta$ Values $[\delta_{(-)} - \delta_{(+)}]$ for MTPA esters (**4a**,**b**, **5a**,**b** and **11a**,**b**) of compounds **4**, **5** and **11** and spatial consequences.

Table 3
^{1}H NMR spectral data of compounds **6**–**8** ($CDCl_3$, 400 MHz)[a,b]

H	6	7	8
1	4.11 (*d*, 6.7)	4.15 (*d*, 6.8)	4.15 (*d*, 6.8)
2	5.40 (*t*, 6.7)	5.43 (*t*, 6.8)	5.40 (*t*, 6.8)
3	–	–	–
4	2.08–2.11 (*m*)	2.73 (*d*, 5.2)	2.33 (*t*,7.0)
5	1.61–1.63 (*m*)	5.57 (*dt*, 15.4, 5.2)	2.81 (*t*, 7.0)
6	4.03 (*t*, 6.3)	5.62 (*d*, 15.4)	–
7	–	–	–
8	2.11–2.14 (*m*)	1.61 (*m*)	2.31 (*t*, 7.0)
9	2.24 (*m*)	2.16 (*m*)	2.17 (*m*)
10	5.22 (*t*, 6.3)	5.25 (*t*, 6.8)	5.22 (*t*, 6.8)
11	–	–	–
12	3.01 (*s*)	3.03 (*s*)	3.03 (*s*)
13	–	–	–
14	6.07 (*s*)	6.09 (*s*)	6.10 (*s*)
15	–	–	–
16	1.84 (*s*)	2.13 (*s*)	1.88 (*s*)
17	2.10 (*s*)	1.87 (*s*)	2.14 (*s*)
18	1.59 (*s*)	1.60 (*s*)	1.60 (*s*)
19	a: 5.02 (*s*)	1.28 (*s*)	a: 6.00 (*s*)
	b: 4.84 (*s*)		b: 5.74 (*s*)
20	1.65 (*s*)	1.66 (*s*)	1.69 (*s*)

[a] δ in ppm (TMS as internal standard), coupling constants (J in parentheses) are given in Hz.
[b] Assignments were confirmed by decoupling and 2D NMR experiments (COSY ^{1}H–^{1}H, ^{1}H J-resolved, HMQC and HMBC).

Table 4
^{13}C NMR spectral data of compounds **6**–**8** ($CDCl_3$, 100 MHz)[a,b]

C	6	7	8
1	59.2 (*t*)	59.3 (*t*)	59.2 (*t*)
2	123.6 (*d*)	124.2 (*d*)	124.1 (*d*)
3	139.3 (*s*)	138.2 (*s*)	138.5 (*s*)
4	35.6[c] (*t*)	42.1 (*t*)	33.9 (*t*)
5	33.4[c] (*t*)	125.2 (*d*)	36.3 (*t*)
6	74.8 (*d*)	138.7 (*d*)	201.1 (*s*)
7	151.3 (*s*)	72.9 (*s*)	148.0 (*s*)
8	30.9 (*t*)	42.3 (*t*)	30.8 (*t*)
9	26.7 (*t*)	23.1[f] (*t*)	27.0 (*t*)
10	128.8[d] (*d*)	129.3[d] (*d*)	128.4[d] (*d*)
11	130.0 (*s*)	129.8 (*s*)	130.6 (*s*)
12	55.1 (*t*)	55.1 (*t*)	55.2 (*t*)
13	199.8 (*s*)	199.3 (*s*)	199.4 (*s*)
14	122.9[d] (*d*)	122.9[d] (*d*)	122.9[d] (*d*)
15	156.0 (*s*)	155.9 (*s*)	155.6 (*s*)
16	27.7[e] (*q*)	27.7[e] (*q*)	27.7[e] (*q*)
17	20.7[e] (*q*)	20.7[e] (*q*)	20.7[e] (*q*)
18	16.5 (*q*)	16.4 (*q*)	16.5 (*q*)
19	110.0 (*t*)	28.2[f] (*q*)	124.1 (*q*)
20	16.3 (*q*)	16.3 (*q*)	16.2 (*q*)

[a] δ in ppm (TMS as internal standard).
[b] Multiplicities were obtained with DEPT sequences. Assignments were made with the aid of HSQC and HMBC experiments.
[c,d,e,f] Each pair of values had been exchanged in comparison to previous assignment (Amico et al., 1981).

As shown in Table 5, *B. bifurcata* and *C. brachycarpa* showed a similar diterpenic composition of their lipidic extracts. In a biological point of view, the close chemical resemblance between these two species does not seem to be due to the occurence of a common ancestor in philogenetic processes but more probably to a same response against fouling or grazing organisms which have similar chemical receptors (Prof. Giaccone, University of Catania, personal communication).

Table 5
Diterpenic composition of *B. bifurcata*[a] and *C. brachycarpa*[b]

Compound	*B. bifurcata*	*C. brachycarpa*
1	×	–
2	×	–
3	×	–
4	×	–
5	×	–
6	×	×
7	×	×
8	×	×
9a	×	×
9b	–	×
10	×	–
11	×	×
12a	×	×
12b	–	×
13	–	×
14	×	×
15	×	×
16a	–	×
16b	–	×
17	×	×

[a] Only compounds described in this paper and/or also isolated from *C. brachycarpa* were presented (for a complete list see Culioli et al., 2004, and references cited therein).
[b] All metabolites isolated from *C. brachycarpa*, under various erroneous names such as *C. crinita*, *C. balearica*, *C. brachycarpa* var. *balearica*, were shown (Amico et al., 1980, 1981, 1987, 1988; Della Pieta et al., 1993).

3. Experimental

3.1. General

Mass spectra (EIMS, 70 eV) were run with a Hewlett–Packard 5972A mass spectrometer coupled to a Hewlett–Packard 5890A gas chromatograph. HRE-IMS spectra were carried out at 70 eV with a Varian MAT 311 double-focusing mass spectrometer with reversed Nier-Johnson BE geometry. IR spectra were recorded with a Jasco Model J-230 FT-IR spectrometer as KBr plates (films). NMR spectra were recorded on a Bruker Avance 400 MHz instrument. Chemical shifts are quoted in ppm (δ) relative to TMS and coupling constants are in Hz. Optical rotations were measured on a Perkin–Elmer 341 polarimeter, using a 10-cm microcell. Silica gel chromatography was performed using Merck Kieselgel 60 powder. Final purification of all new metabolites was achieved by HPLC on a C-18 reverse-phase column (250 × 4 mm, Biotek Peakmax, 4 μm) with RI monitoring.

3.2. Plant material

Bifurcaria bifurcata (Velley) Ross was collected near Quiberon (47°29′N; 3°7′O), Brittany, France, in July 1999. A voucher specimen of this species (CO-171) is deposited in the Herbarium Crouan (Département de Biologie Marine, Muséum National d'Histoire Naturelle, Concarneau, France).

3.3. Extraction and purification

The shade-dried material collected from Quiberon (900 g) was ground and extracted with $CHCl_3$–MeOH (1:1) at room temperature. After filtration, the filtrate was evaporated to yield 28 g of crude extract. This extract was subjected to CC on silica gel with a solvent gradient from cyclohexane to EtOAc and then from EtOAc to MeOH. The fractions eluted with cyclohexane–EtOAc (1:1) contained sterols, eleganolone (compound **9a** (3.62 g)) together with diterpenes previously described (compounds **10** (120 mg), **12a** (72 mg) and **17** (45 mg)) from this species (Culioli et al., 1999a,b, 2000). Those eluted with cyclohexane–EtOAc (2:3) contained eleganediol (compound **11** (1.35 g)) and for the last ones, compounds **1**–**3** and **6**–**8**. The two new compounds (**4** and **5**) were eluted with cyclohexane–EtOAc (1:4). All these fractions were further purified several times by HPLC on an analytical C-18 reverse-phase column (MeCN–H_2O, (1:1) for **1**–**3** and **6**–**8**, and MeCN–H_2O (2:3) for **4** and **5**) to give **1** (3 mg), **2** (4 mg), **3** (1 mg), **4** (1 mg), **5** (1 mg), **6** (5 mg), **7** (5 mg) and **8** (1 mg).

3.4. Compound *1*

Oil; IR ν_{max}^{film} cm^{-1}: 3522, 2975, 2929, 2857, 1703, 1679, 1442, 1387, 1376, 1150, 998; HRMS: *m/z* 322.2511 $[M]^+$ (calc. for $C_{20}H_{34}O_3$, *m/z* 322.2508); EIMS (70 eV) *m/z* (rel. int.): 322 $[M]^+$ (0.3), 286 $[M - 2H_2O]^+$ (0.3), 253 (0.5), 137 (9), 136 (9), 95 (1), 83 (53), 69 (100), 55 (9). For ^{13}C and ^1H NMR spectra, see Tables 1 and 2.

3.5. Compound *2*

Oil; $[\alpha]_D^{25} + 3°$ ($CHCl_3$; *c* 0.52); IR ν_{max}^{film} cm^{-1}: 3466, 2974, 2932, 2858, 1687, 1619, 1445, 1383, 1050, 908; HRMS: *m/z* 320.2353 $[M]^+$ (calc. for $C_{20}H_{32}O_3$, *m/z* 320.2352); EIMS (70 eV) *m/z* (rel. int.): 320 $[M]^+$. (0.4), 302 $[M - H_2O]^+$ (0.1), 289 (0.1), 201 (0.1), 151 (2), 138 (2), 99 (10), 95 (6), 93 (3), 83 (100), 81 (6), 73 (5), 71 (6), 69 (6), 55 (19), 43 (34). For ^{13}C and ^1H NMR spectra, see Tables 1 and 2.

3.6. Compound *3*

Oil; $[\alpha]_D^{25} + 12°$ ($CHCl_3$; *c* 0.25); IR ν_{max}^{film} cm^{-1}: 3401, 2962, 2919, 2867, 1711, 1620, 1444, 1380, 1053; HRMS: *m/z* 322.2501 $[M]^+$ (calc. for $C_{20}H_{34}O_3$, *m/z* 322.2508); EIMS (70 eV) *m/z* (rel. int.): 322 $[M]^+$ (0.2), 286 $[M - 2H_2O]^+$ (0.1), 211 (4), 188 (7), 121 (25), 107 (15), 93 (13), 85 (100), 81 (12), 68 (28), 57 (59). For ^{13}C and ^1H NMR spectra, see Tables 1 and 2.

3.7. Compound *4*

Oil; $[\alpha]_D^{25} - 3°$ ($CHCl_3$; *c* 0.15); IR ν_{max}^{film} cm^{-1}: 3369, 2971, 2917, 2866, 1447, 1375, 1051, 1008; HRMS: *m/z* 322.2510 $[M]^+$ (calc. for $C_{20}H_{34}O_3$, *m/z* 322.2508); EIMS (70 eV) *m/z* (rel. int.): 322 $[M]^+$ (0.1), 268 $[M - 3H_2O]^+$ (0.1), 220 (0.6), 207 (0.4), 149 (6), 119 (21), 107 (26), 93 (31), 85 (100), 69 (30), 55 (40). For ^{13}C and ^1H NMR spectra, see Tables 1 and 2.

3.8. Compound *5*

Oil; $[\alpha]_D^{25} - 7°$ ($CHCl_3$; *c* 0.20); IR ν_{max}^{film} cm^{-1}: 3394, 2970, 2938, 2875, 1715, 1670, 1444, 1386, 1152, 1050, 1011; HRMS: *m/z* 322.2505 $[M]^+$ (calc. for $C_{20}H_{34}O_3$, *m/z* 322.2508); EIMS (70 eV) *m/z* (rel. int.): 322 $[M]^+$ (0.1), 304 286 $[M - H_2O]^+$ (0.1), 286 $[M - 2H_2O]^+$ (0.1), 219 (5), 186 (7), 168 (13), 119 (22), 107 (18), 93 (38), 85 (96), 69 (15), 57 (100). For ^{13}C and ^1H NMR spectra, see Tables 1 and 2.

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