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(*S*)-13-HYDROXYGERANYLGERANIOL-DERIVED FURANODITERPENES FROM *BIFURCARIA BIFURCATA*

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Key Word Index—*Bifurcaria bifurcata*; Cystoseiraceae; brown alga; diterpenes; (*S*)-13-hydroxygeranylgeraniol; furane; epoxylactone; antimitotic; geographical variation.

Abstract—Two new diterpenes were isolated from a sample of the brown alga *Bifurcaria bifurcata* collected from Brittany on the Atlantic coast and their structures established by spectroscopic methods. The new diterpenes are derived from (*S*)-13-hydroxygeranylgeraniol by terminal cyclization and oxidation leading to a furan-3-yl ring or a β,γ-epoxy-γ-lactone. One of them showed a cytotoxic effect to fertilized sea urchin eggs. The chemical shifts of the methyl groups and quaternary carbons in (*S*)-13-hydroxygeranylgeraniol have been revised to take account of the results obtained in a 2D NMR long-range C–H correlation experiment and the absolute configuration at C-13 determined for the first time. The geographical variation in the diterpenoid composition of *B. bifurcata* was also studied.

INTRODUCTION

As part of a photochemical study of the Atlantic brown alga *Bifurcaria bifurcata* (Phaeophyceae) [1–4], including the geographical variation of its diterpenoid composition [4], we describe the isolation and structure elucidation of two new furanoditerpenes derived from (*S*)-13-hydroxygeranylgeraniol (**1**), a previously reported acyclic diterpene which was named eleganediol by its discoverers [5]. These compounds, together with **1**, have been isolated from species collected on the French Atlantic coast. One of them showed a cytotoxic effect to fertilized sea urchin eggs. This activity was compared to that of previously described acyclic diterpenes [2–4].

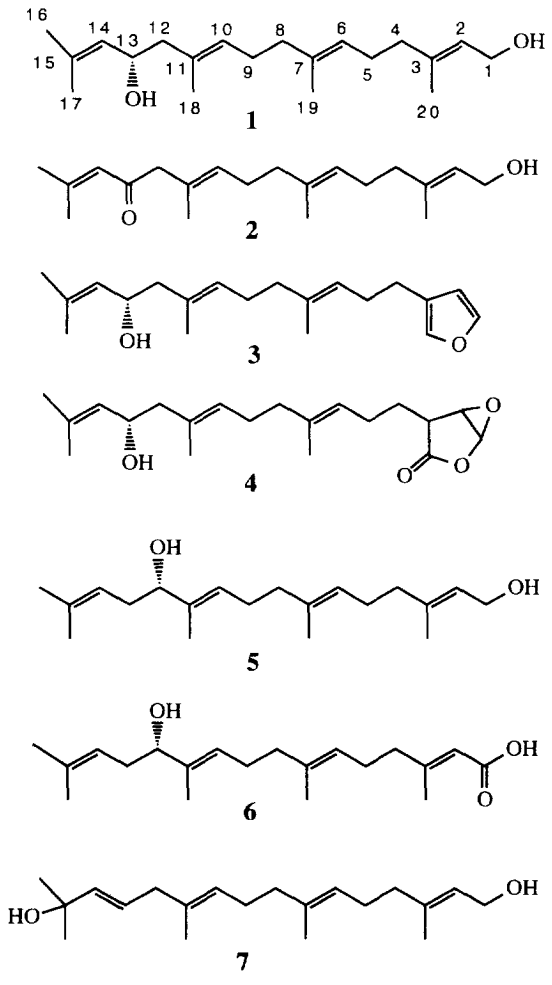
Furanoterpenoids (sesqui-, di- and mainly sesterterpenes) are known to be specific to sponges [6, 7]. Three related metabolites have been isolated from *B. bifurcata* [8], but in this case they were derived from elganolone (**2**), a previously described acyclic diterpene present in *B. bifurcata* [5] as well as in *Cystoseira* species [9].

RESULTS AND DISCUSSION

The ether extract of dried *B. bifurcata* collected near Roscoff (France) was fractionated by liquid chromatography using silica gel. The fraction eluted with hexane–ether (1:1) was further purified by HPLC on normal phase silica. From this separation, we obtained the previously reported acyclic diterpene **1** as well as two new compounds (**3** and **4**).

Diterpene **3** (bifurcane), $C_{20}H_{30}O_2$ (HRMS), is an optically active oil (0.23% dry wt) which showed hydroxyl absorption (3400 cm^{-1}) in its IR spectrum. Its EI-mass spectrum exhibited a base peak at *m/z* 85 (100%) corresponding to cleavage of the C-12/C-13 bond as in eleganediol [5]. The ^1H and ^{13}C NMR data were similar to those of **1** except for the signals corresponding to the first isoprenoid unit (Tables 1 and 2). In particular, the characteristic ^1H NMR signal of H-13 was observed. Irradiation of **1** successively at δ2.09, 4.37 and 5.16 showed that this signal was a *ddd* ($^3J_{H_{13}-H_{14}} = {}^3J_{H_{13}-H_{12a}} = 8.2$ Hz and $^3J_{H_{13}-H_{12b}} = 5.3$ Hz) and not a *dt* as described in ref. [5]. The position of the C-18 and C-19 methyl signals above δ20 (Table 2) verified the all *E* configuration of the double bonds of the acyclic isoprenoid chain [10, 11] in **3** and **4**. A Horeau determination [12] of the absolute configuration of the alcohol indicated (*S*) configuration for the hydroxyl-bearing C-13 of compounds **1**, **3** and **4**.

In the course of comparing the acyclic isoprenoid chain of compounds **1**, **3** and **4**, the location of methyl and quaternary carbons in **1** had to be revised to take account of the results obtained in a 2D NMR long-range C–H correlation experiment (COLOC) in C_6D_6. These showed that the NMR data pertaining to C-3 and C-15



in **1** should be exchanged in ref. [5] as well as those of the methyl groups (Tables 1 and 2).

The presence of a furanyl ring in **3** was indicated firstly by the absorptions at 875 and 780 cm^{-1} in its IR spectrum and secondly, by the ^1H NMR signals at δ7.34, 7.21 and 6.28 [8, 13]. This was confirmed by the ^{13}C NMR assignments (three olefinic methine carbons at δ142.5, 138.9 and 111.1 and one olefinic quaternary carbon at δ125.0 [14]) coupled to homo- and hetero-nuclear 2D NMR experiments (COSY ^1H–^1H, XHCORR and HMBC). In particular, the heteronuclear multi-bond connectivities between C-3/H-2, C-3/H-4 and C-3/H-20 ($^2J_{C-H}$ chemical shift correlations) were very important because they enabled us to specify that the furanyl ring was linked to the acyclic isoprenoid moiety by C-3.

Diterpene **4** (epoxyeleganolactone), $C_{20}H_{30}O_4$ (HRMS), is an optically active oil (0.03% dry wt) which showed hydroxyl absorption (3400 cm^{-1}) in its IR spectrum and a base peak *m/z* 85 (100%) in its EI-mass spectrum as in the cases of **1** and **3**. The ^1H and ^{13}C NMR data of its acyclic moiety were similar to those of **1** and **3** (Tables 1 and 2), as well as the configuration at the C-13 (Horeau determination) and at the isoprenoid double bonds (C-18 and C-19 methyls signals above δ20). In this case, the first isoprenoid unit includes an α-substituted-β,γ-epoxy-γ-lactone group which was revealed by: (i) the IR absorptions at 1774 and 1250 cm^{-1} (ii) the ^1H NMR signals at δ5.58 (*d*, J = 2.4 Hz), 3.80 (*t*, J = 2.5 Hz) and 2.82 (*ddd*, J = 10.0, 5.0 and 2.5 Hz) in $CDCl_3$ [8, 15] which were strongly upfield shifted in C_6D_6, at δ4.70, 2.86 and 2.07 (Table 1) respectively, and (iii) the ^{13}C NMR assignments in $CDCl_3$ of two oxygenated sp^3 methines at δ77.6 and 53.7, one non-oxygenated sp^3 methine at δ43.1 and a quaternary carbon corresponding to the lactone C=O at δ175.6 [15] (Table 2). These assignments were confirmed by homo- and hetero-nuclear 2D NMR experiments (^1H–^1H COSY, XHCORR and HMBC). The main long-range ^2J, $^3J_{C-H}$ chemical shift correlations were the C-20/H-1, C-20/H-2 connectivities as well as those of C-3/H-4, C-2/H-4 and C-20/H-4. These last correlations enabled us to specify that the β,γ-epoxy-γ-lactone moiety was linked to the acyclic chain by C-3.

We think that epoxyeleganolactone (**4**) could be an oxidation product of bifurcane (**3**) as has been shown by the autoxidation studies on the marine furanosesterterpene variabilin [15]. In this case, the singlet oxygen autoxidation products of the 3-alkyl furan moiety of the molecule include β,γ-epoxy-γ-lactones originating via thermal rearrangements of an initially formed hydroperoxide. Mechanistic considerations resulting from the oxidation pathway of 3-alkyl furans [15] connected with the value of the coupling constants of H-1, H-2 and H-3 (*ca* 2.5 Hz) in ^1H NMR signals, led us to propose that the stereochemistry of the epoxide functionality and H-3 must be *trans* in **4**. This hypothesis was confirmed by a ^1H–^1H NOE difference spectroscopy experiment which showed an enhancement between H-2 and H-3 (3%). However, **4** does not seem to be a diastereoisomeric mixture, but an optically active pure compound, as was shown by ^1H and ^{13}C NMR data and by its $[\alpha]_D$ significant value ($-$ 9.1°); even after oxidation at C-13 which leads to the eleganolone derivative.

Cytotoxic activity. Sea urchin egg development is frequently used as a pharmacological screen for compounds that inhibit cell division [16]. The activity of one of the two new metabolites (**3**) was compared to that of compounds **1**, **5**, **6** and **7** previously reported in ref. [4]. The ED_{50} value for inhibition of development of fertilized eggs of the common sea urchin *Paracentrotus lividius* were as follows. Compounds **3** and **7** were active at the lowest concentrations (12 and 4 $\mu g\,ml^{-1}$, respectively). Compounds **1**, **5** and **6** were active at 36, 18 and 60 $\mu g\,ml^{-1}$, respectively.

Geographical variations. To complete the study of the geographical variations in the diterpenoid composition of *B. bifurcata*, limited to the Moroccan Atlantic coasts in our previous paper [4], we have collected this species on the French Atlantic coasts, from Vannes in the south to Saint-Brieuc in the north. The different collections were treated and extracted in an identical fashion [4]. Each ether extract was analysed by HPLC (ethyl acetate–isooctane, 3:2) to determine its diterpenoid composition.

Table 1. ^{1}H NMR spectral data for compounds **1**, **3** and **4** (TMS as int. standard)*

	1 (360 MHz)	**1** (200 MHz)	**3** (200 MHz)	**4** (200 MHz)	
H	$CDCl_3$	C_6D_6	$CDCl_3$	$CDCl_3$	C_6D_6
1	4.10 *d* (6.7)	4.11 *d* (6.6)	7.34 *m*	5.58 *d* (2.4)	4.70 *d* (2.5)
2	5.42 *t* (6.7)	5.50 *t* (6.6)	6.28 *m*	3.80 *t* (2.5)	2.86 *m*
				2.82 *ddd* (10.0, 5.0, 2.5)	2.07 *m*
4	2.00 *m*	2.04 *m*	2.47 *t* (7.3)	1.90–1.78 *m*	1.82–1.69 *m*
5	2.08 *m*	2.12 *m*	2.24 *m*	2.28 *m*	2.02 *m*
6	5.12 *t* (6.7)	5.22 *t* (6.7)	5.17 *t* (7.0)	5.16 *t* (6.8)	5.01 *t* (7.0)
8	2.00 *m*	2.04 *m*	2.03 *m*	2.03 *m*	2.02 *m*
9	2.08 *m*	2.18 *m*	2.12 *m*	2.14 *m*	2.12 *m*
10	5.18 *t* (6.7)	5.25 *t* (6.7)	5.21 *t* (7.0)	5.21 *t* (6.8)	5.22 *t* (6.7)
12	2.09 *m*	2.21–2.29 *m*	2.13 *m*	2.10 *m*	2.24 *m*
13	4.37 *ddd* (8.2, 8.2, 5.3)	4.48 *ddd* (8.2, 8.2, 5.1)	4.41 *ddd* (8.3, 8.3, 5.0)	4.40 *ddd* (8.2, 8.2, 8.2, 5.3)	4.45 *ddd* (8.3, 8.3, 5.5)
14	5.16 *d* (8.2)	5.32 *d* (8.3)	5.16 *d* (8.3)	5.15 *d* (8.2)	5.29 *d* (8.3)
16	1.69 *s*	1.63	1.72 *s*	1.72 *s*	1.61 *s*
17	1.66 *s*	1.58	1.69 *s*	1.69 *s*	1.56 *s*
18	1.62 *s*	1.60	1.66 *s*	1.66 *s*	1.59 *s*
19	1.57 *s*	1.56	1.59 *s*	1.65 *s*	1.47 *s*
20	1.64 *s*	1.54	7.21 *s*	–	–

*Coupling constants (*J* in parentheses) are given in Hz; assignments were confirmed by decoupling and 2D NMR experiments (^{1}H–^{1}H COSY, XHCORR and HMBC); HMBC spectra were recorded at 400 MHz on a different machine.

Table 2. ^{13}C NMR spectral data for compounds **1**, **3** and **4** (TMS as int. standard)*

	1			**3**		**4**		
C	$CDCl_3$†	DEPT	C_6D_6‡	$CDCl_3$‡	DEPT	$CDCl_3$‡	C_6D_6‡	DEPT
1	59.3	CH_2	59.3	142.5	CH	77.6	78.2	CH
2	124.3	CH	125.3	111.1	CH	53.7	54.3	CH
3	139.4	C	137.6	125.0	C	43.1	43.8	CH
4	39.5	CH_2	39.9	25.0	CH_2	26.8	27.9	CH_2
5	25.8	CH_2	26.7	28.4	CH_2	25.8	26.7	CH_2
6	123.6	CH	124.9	124.2	CH	123.0	124.5	CH
7	134.8	C	134.9	134.9	C	134.9	134.5	C
8	39.4	CH_2	39.8	39.5	CH_2	39.5	40.6	CH_2
9	26.2	CH_2	26.5	26.4	CH_2	26.4	27.5	CH_2
10	127.4	CH	128.2	127.5	CH	128.2	129.1	CH
11	131.6	C	132.1	131.7	C	131.9	133.3	C
12	48.2	CH_2	48.7	48.2	CH_2	48.2	49.5	CH_2
13	65.6	CH	66.5	65.6	CH	65.7	67.2	CH
14	128.5	CH	129.1	128.5	CH	127.5	129.9	CH
15	135.0	C	133.5	135.5	C	137.0	137.4	C
16	26.4	CH_3	25.8	25.8	CH_3	25.8	26.6	CH_3
17	18.2	CH_3	18.2	18.2	CH_3	18.2	19.0	CH_3
18	16.3	CH_3	16.4	16.2	CH_3	16.2	17.2	CH_3
19	15.9	CH_3	15.9	15.9	CH_3	16.0	16.7	CH_3
20	16.2	CH_3	16.2	138.9	CH	175.6	175.3	C

*Multiplicities were obtained with DEPT sequences.
†Measured at 90 MHz.
‡Measured at 50 MHz.

Table 3. Geographical variations in the diterpenoid composition of *B. bifurcata*

Compounds (mg g^{-1} algal dry wt)										
Rabat*		El Jadida*			Oualidia*		Roscoff†		Saint-Brieuc†	
1	**7**	**1**	**2**	**7**	**5**	**6**	**1**	**3**	**1**	**2**
3.73	0.50	1.01	3.06	0.14	3.33	0.88	1.52	2.34	1.45	2.54

*Data of ref. [4] added for comparison (July collection).
†August collection.

The results listed in Table 3 show that two 'chemical types' of *B. bifurcata* can be clearly defined. Type 1, with **2** as the main diterpenoid, was obtained from two zones of collection: Lorient (between Piriac sur mer and Melon) and Saint-Brieuc (between Lannion and Cap de La Hague); while type 2, with **3** as the main diterpenoid, was obtained from the Roscoff zone (between Melon and Lannion). The last one is a new chemical type which is not present on the Moroccan coasts [4].

This work has shown that the French collections contained only compounds directly or indirectly derived from hydroxylation of geranylgeraniol at C-13 (**1**–**4**). By contrast, the Moroccan collections contained compounds derived from geranylgeraniol by hydroxylation at C-12 or C-13 (**1**, **2**, **5**–**7**), leading to three chemical types [4] including the precedent type 1.

EXPERIMENTAL

General. MS: direct inlet, 70 eV; ^{1}H NMR: 200, 360 and 400 MHz; ^{13}C NMR: 50, 90 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 (Intersphere Si-60, 5 μm), with RI monitoring.

Plant material. Bifurcaria bifurcata Ross was collected near Roscoff, France in July–August 1992 for isolation of compounds **1**, **3** and **4**, and in August 1992 for the study of geographical variations in diterpenoid composition (French Atlantic coasts). A voucher specimen of this species is deposited in the Herbarium of Dr Pellegrini, Laboratoire de Biologie Fondamentale et Appliquée, University of Marseille II, France.

Extraction and purification. The freeze-dried material (100 g) was ground and extracted with Et_2O at room temp. After filtration and evapn, the extract was partitioned between H_2O and Et_2O. The Et_2O-soluble material was dried over $MgSO_4$ and the filtrate was evapd to yield 2.4 g of a crude extract which was subjected to CC on silica gel eluted with a solvent gradient from hexane to EtOAc. The two new compounds and eleganediol (**1**) were eluted with hexane–EtOAc (1:1) and subsequently purified by HPLC (EtOAC–iso-octane, 3:2) to give **1**, (152 mg), **3** (234 mg) and **4** (30 mg).

Bifurcane (**3**). Oil; $[\alpha]_D^{25}$ − 7.6° (EtOH; *c* 9.5); IR ν_{max}^{film} cm^{-1}: 3400, 2900, 1440, 1380, 1150, 1020, 875, 780; HRMS: 284.2143 $[M - H_2O]^+$ (calc. for $C_{20}H_{28}O$, 284.2140); EIMS (70 eV) *m/z* (rel. int.): 284 $[M - H_2O]^+$ (9), 218 (10), 203 (8), 175 (10), 135 (21), 107 (15), 93 (23), 85 (100), 81 (58), 69 (37), 43 (99); ^{1}H and ^{13}C NMR: Tables 1 and 2.

Epoxyeleganolactone (**4**). Oil; $[\alpha]_D^{25}$ − 9.1° (CH_2Cl_2; *c* 2.2); IR ν_{max}^{film} cm^{-1}: 3400, 2900, 1774, 1452, 1380, 1250, 1120, 1028, 890, 844; HRMS: 316.2042 $[M - H_2O]^+$ (calc. for $C_{20}H_{28}O_3$, 316.2038); EIMS (70 eV) *m/z* (rel. int.): 334 $[M]^+$ (0.2), 316 $[M - H_2O]^+$ (0.4), 218 (15), 203 (14), 175 (12), 136 (12), 123 (9), 94 (13), 85 (100), 81 (51), 69 (27); ^{1}H and ^{13}C NMR: Tables 1 and 2.

HPLC analysis of compounds **1**–**3**. The method previously described for the determination of sterols and diterpenoids from Cystoseiraceae [17] was used. The int. standard was replaced by 2-hydroxyacetophenone in the case of **3**.

Absolute configuration at C-13. The alcohol (15 mg) in dry pyridine (150 μl) was treated with (±)-2-phenylbutyric anhydride (50 mg) and left overnight at room temp. H_2O (500 μl) was added and the mixt. warmed for 30 min until a homogeneous soln obtained. H_2O (2 ml) and C_6H_6 (3 ml) were added and the mixt. titrated with NaOH (0.1 M) until alkaline (phenolphthalein). C_6H_6 (10 ml) was added and the layers sepd. 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 concd. The observed rotations were: − 0.05 (reaction with **1**), − 0.03 (reaction with **3**) and − 0.05 (reaction with **4**). As the acid excess was laevorotatory, compounds **1**, **3** and **4** had the 13(*S*) configuration.

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REFERENCES

1. Combaut, G. and Piovetti, L. (1983) *Phytochemistry* **22**, 1787.
2. Valls, R., Banaigs, B., Francisco, C., Codomier, L. and Cave, A. (1986) *Phytochemistry* **25**, 751.

3. Semmak, L., Zerzouf, A., Valls, R., Banaigs, B., Jeanty, G. and Francisco, C. (1988) *Phytochemistry* **27**, 2347.
4. Valls, R., Banaigs, B., Piovetti, L., Archavlis, A. and Artaud, J. (1993) *Phytochemistry* **34**, 1585.
5. Biard, J. F., Verbist, J. F., Floch, R. and Letourneux, Y. (1980) *Tetrahedron Letters* **21**, 1849.
6. Faulkner, D. J. (1992) *Nat. Prod. Rep.* **9**, 323.
7. Schmitz, F. J., Bowden, B. F. and Toth, S. I. (1993) in *Marine Biotechnologies* (Attaway, D. H. and Zaborsky, O. R. eds), Vol. I, p. 197. Plenum Press, New York.
8. Houggard, L., Anthoni, U., Christophersen, C. and Nielsen, P. H. (1991) *Phytochemistry* **30**, 3049.
9. Francisco, C., Combaut, G., Teste, J. and Prost, M. (1978) *Phytochemistry* **17**, 1003.
10. Couperus, P. A., Clague, A. D. H. and Van Dongen, J. P. C. M. (1976) *Org. Magn. Reson.* **8**, 426.
11. Coates, R. M., Ley, D. A. and Cavender, P. L (1978) *J. Org. Chem.* **43**, 4915.
12. Horeau, A. and Nouaille, A. (1971) *Tetrahedron Letters* **12**, 1939.
13. Garcia, M. O. and Rodriguez, A. D. (1990) *Tetrahedron* **46**, 1119.
14. D. Giulio, A., De Rosa, S., Di Vicenzo, G. and Zovodnik, N. (1989) *J. Nat. Prod.* **52**, 1258.
15. Barrow, C. J., Blunt, J. W. and Munro, M. H. G. (1989) *J. Nat. Prod.* **52**, 346.
16. Jacobs, R. S., White, S. and Wilson, L. (1981) *Fred. Proc. Am. Soc. Exp. Biol.* **40**, 26.
17. Piovetti, L., Deffo, P., Valls, R. and Peiffer, G. (1991) *J. Chromatogr.* **588**, 99.