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A NOVEL ACYCLIC DITERPENE FROM THE BROWN ALGA *BIFURCARIA BIFURCATA*

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Key Word Index—*Bifurcaria bifurcata*; Cystoseiraceae; brown algae; acyclic diterpene; 7,8-epoxy-1-hydroxy-13-keto-3,7,11,15-tetramethylhexadeca-2*E*,5*E*,10*E*,14-tetraene.

Abstract—A novel epoxy-diterpene identified as 7,8-epoxy-1-hydroxy-13-keto-3,7,11,15-tetramethylhexadeca-2*E*,5*E*,10*E*,-14-tetraene was isolated from *Bifurcaria bifurcata*, a brown alga belonging to the family Cystoseiraceae, in which eleganolone, epoxy-eleganolone and eleganediol have previously been isolated.

INTRODUCTION

Among the brown algae of the family Cystoseiraceae, the genera *Halidrys* [1], *Cystophora* [2], *Cystoseira* [3–11] and *Bifurcaria* [12, 13] have been studied for biologically active natural products. Some acyclic diterpenes have been identified from *Cystoseira elegans* and *Cystoseira crinita* [5–10], some monomethyl hydroquinols with oxygenated side chains from *Halidrys siliquosa* [1] and *Cystoseira stricta* [11], and some resorcinol and phloroglucinol derivatives have been isolated from *Cystophora torulosa* [2]. The monocyclic diterpenoid, **1**, has been isolated from the endemic *Bifurcaria* species of the Galapagos Islands [12] and the acyclic diterpenoids **2**–**4** from the Atlantic French coast *Bifurcaria bifurcata* [13]. These last structures are related to eleganolone (**3**) which was first isolated from *Cystoseira elegans* [6] and it is also present in *Cystoseira crinita* [7]. We report now the isolation and structural elucidation of an acyclic diterpene from *Bifurcaria bifurcata* assigned as **5a**.

RESULTS

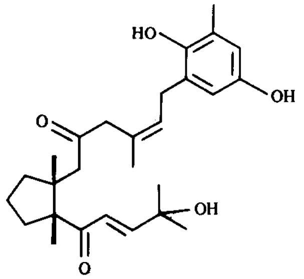
The acyclic diterpene, **5a**, was isolated from *B. bifurcata* as a brown oil after open Si gel CC of the ether extract of the dried alga, using hexane with increasing amounts of ether as eluant. The more polar fraction was purified by HPLC, using dichloromethane–ethyl acetate (90:10) as eluant, to give pure **5a** as a viscous oil. The IR spectrum (carbon tetrachloride) showed absorptions for hydroxyl (3430 cm^{-1}), α,β-unsaturated ketone (1680 cm^{-1}) and olefin (1620 cm^{-1}) functionalities. The UV spectrum (chloroform) consisted of an absorption at 248 nm (ε 12 000) characteristic of the α,β-unsaturated ketone moiety. Acetylation of **5a** (acetic anhydride–pyridine) gave an acetate which lacked IR hydroxyl absorption, excluding the possibility of a tertiary hydroxyl group. The ^{1}H NMR, ^{13}C NMR and mass spectra were assigned by comparison with the spectra of eleganolone (**3**). The ^{1}H NMR spectrum of **5a** was similar to that of **3**: δ 6.09 (1H, *s*, H-14) 4.08 (2H, *d*, $J = 6.7$ Hz, H-1) 2.98 (2H, *s*, H-12) 1.87 (3H, s, H-16). Some novel chemical shifts were observed: δ 1.27 (3H, *s*) 2.72 (2H, *d*, $J = 5$ Hz) 3.55 (1H, *dd*, $J = 6.7$, 12 Hz) 5.17 (1H, *m*) 5.44 (1H, *d*, $J = 15.6$ Hz). Some unchanged chemical shifts appeared with modified integrations: δ 5.42 (*t*, $J = 6.7$ Hz) and 1.62 for two and six protons, respectively, instead of three and nine for **3** and δ 2.11 representing three protons for **3** and five for **5a**. The signal at δ 2.04 (8H, *s*) disappeared implicating the loss of four methylenes compared to **3**. In the mass spectra peaks at m/z 300 $[\mathrm{M}-18]^+$ for **5a**, and at m/z 282 $[\mathrm{M}-\mathrm{HOAc}]^+$ for the acetate and the base peak at m/z 83.0497 $[\mathrm{C_5H_7O}]^+$ suggested **5a** was a $C_{20}H_{30}O_3$ diterpene related to eleganolone (**3**).

The 3H signal at δ 1.27 was assigned as a tertiary methyl deshielded by an adjacent oxygen function. Since complete acetylation of **5a** excluded a tertiary hydroxyl group, an epoxide function was indicated. The position of the epoxide was indicated by an ion at m/z 208.150 (**5b**, $C_{13}H_{20}O_2$ calculated 208.146) resulting from a proton transfer.

A mass spectral fragment at m/z 167 (**5c**, $C_{10}H_{15}O_2$) and 149 ($C_{10}H_{13}O$) suggested the cleavage A and $[\mathrm{A}-\mathrm{H_2O}]^+$ which allowed the final structure of **5a** to be proposed.

This structure explains by comparison with **3** the absence of the four methylenes at δ 2.04, two of which were shifted to 2.11 and 2.72. The latter doublet at 2.72 was the X_2 part of an ABX_2 system; the olefinic region which included the AB part integrated for one proton more than in the spectrum of **3**. The vinyl methyl signal at δ 1.62 in **3** was replaced by a signal at 1.27 assigned to the epoxide methyl.

The structure **5a** was confirmed by the ^{13}C NMR spectrum, particularly by the signals for the 7,8-epoxide at δ 61.3, the methyl adjacent to an oxygen function at 22.7 and the C-4 methylene at 41.8. These data can be favorably related to that of the previously described diterpenes **2** and **6** [13, 10]. The ^{13}C NMR data also led to a *trans*-(*E*)-configuration of the double bonds at C-2 and C-10 indicated by the shielded chemical shifts of the methyls at C-3 and C-11 (16.5 and 16.7, respectively) [10]. The stereochemistry of the C-5 double bond was deduced to be *E* from the value of $J_{5,6} = 15.6$ Hz of the ^{1}H NMR spectrum. The stereochemistry of the C-7, C-8 centers can be deduced from the previous ^{1}H NMR data of another epoxide isolated from an algal source, the halogen cham-



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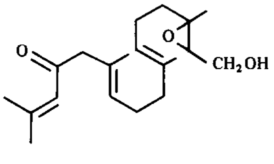

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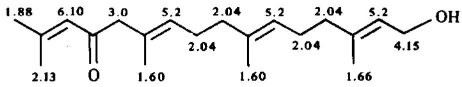

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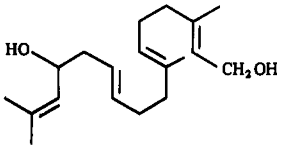

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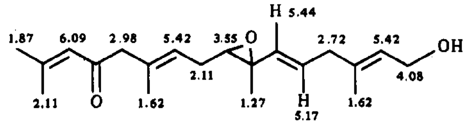
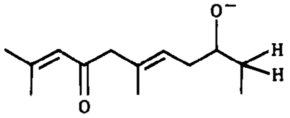


5a



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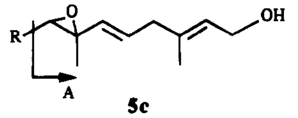

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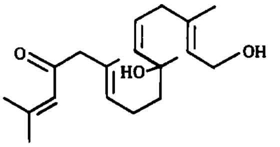

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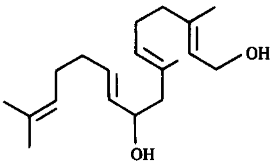

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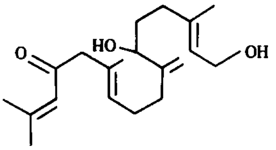

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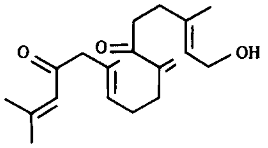

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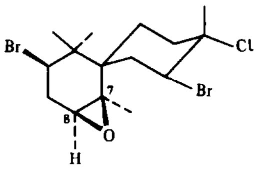

10

igrene derivative **10** [14]. The C-7 and C-8 centers are in a ring and this compound is 7*R*, 8*S*. It showed a δ 1.48 singlet for the tertiary methyl group and a multiplet at 2.93 (*dd*, $J = 3.3$ Hz) for the C-8 proton.

The epoxy-methyl system of **5a** shows, in comparison with **10**, a shielded methyl (1.48–1.27) and a deshielded proton (2.93–3.55) conforming to a *Z*-configuration for the methyl at δ 1.27 and the methylene at 2.11. Therefore, the natural product **5** can correspond to one of the two diasteresisomers 7*R*, 8*R* or 7*S*, 8*S*.

DISCUSSION

A chemical analogy exists between the genera *Bifurcaria* and *Cystoseria*. Further support for this comes from the isolation of acyclic diterpenoids with an α,β-unsaturated ketone moiety with the *S-cis*-conformation from algae belonging to both genera. Regarding the French Mediterranean coasts, *Cystoseira elegans* is the only *Cystoseira* species to contain acyclic diterpenoids [8], while the Sicilian *Cystoseira crinita* contains eleganolone (**3**) as the main diterpenoid with the other related diterpenoids, **6**, **8** and **9**. On the other hand a monomethyl hydroquinol has been isolated from the Sicilian *Cystoseira stricta* [11]. As in the genus *Cystoseira*, the genus *Bifurcaria* produces acyclic diterpenoids (**2**–**4** and **5a**) for the species *Bifurcaria bifurcata*. *B. galapagensis*, on the other hand, contains **1** which possesses a monocyclic diterpenoid moiety in combination with a methyl hydroquinol unit. Thus, some *Cystoseira* and *Bifurcaria* species are able to synthesize acyclic diterpenoids (*C. elegans*, *C. crinita*, *B. bifurcata*) while other species of these genera (*C. stricta*, *B. galapagensis*) are able to synthesize metabolites with diterpenoid and methyl hydroquinol moieties.

EXPERIMENTAL

Extraction and isolation. Bifurcaria bifurcata collected for 1 year near Piriac (French Atlantic coast) was freeze-dried and ground to a fine powder with a blender. The dried alga (900 g) was extracted by 3 × 3 l. Et_2O (3 × 3 hr at 20°). The extract was concd under vacuum to give a dark green oil (33 g). The crude extract was applied to a column of Si gel. The column was eluted with a solvent gradient system from hexane to Et_2O. Eleganolone (**3**) was eluted by hexane–Et_2O (70:30) and the more polar fractions were pooled and subjected to HPLC with CH_2Cl_2–EtOAc

(90:10) to give pure 7,8-epoxy-1-hydroxy-13 keto-3,7,11,15-tetramethylhexadeca-2*E*,5*E*,10*E*,14-tetraene (**5a**, 100 mg); IR $\nu_{\max}^{\mathrm{CCl_4}}$ cm^{-1}: 3430, 1680, 1620; UV $\lambda_{\max}^{\mathrm{CHCl_3}}$ nm: 248 (ε, 12 000); MS *m*/*z*: 300 $[\mathrm{M}-18]^+$, 83.0497 (100%).

^{1}H NMR: Chemical shifts for compounds **3** and **5a** are shown on the structures; ^{13}C NMR: δ 55.2 (C-1), 124.2 (C-2), 139.6 (C-3), 41.8 (C-4), 126.5 (C-5), 141.2 (C-6), 61.3 (C-7), 61.3 (C-8), 33.9 (C-9), 119.1 (C-10), 127.6 (C-11), 55.3 (C-12), 199.5 (C-13), 128.5 (C-14), 156.1 (C-15), 20.7 (C-16), 16.5 (C-17), 22.7 (C-18), 16.7 (C-19), 27.7 (C-20).

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