spectrum exhibited methyl singlets at δ1.24 (H-13), 1.48 (H-14), 1.71 (H-15) and two acetyl methyl groups as singlets at δ2.105 and 2.03. The olefinic protons were at δ5.24 as a double doublet for H-1 and a broadened doublet at δ4.72 for H-5. The double doublets at δ5.11 and 5.17 indicated the protons geminal to acetyl groups. The triple doublet at δ2.37 and double quartet at δ2.73 indicated H-7 and H-11, respectively. The stereochemistry of the acetyl groups were assigned by using Dreiding models and by measuring J values of H-9 ($J_{9\beta,8\alpha} = 5$ Hz; $J_{9\beta,8\beta} = 10.5$ Hz) and H-3 ($J_{3\alpha,2\beta} = 9$ Hz; $J_{3\alpha,2\alpha} = 5.5$ Hz). The ^{1}H NMR data of **1** is given in Table 1.

EXPERIMENTAL

Achillea sintenisii was collected from central Turkey (Sivas). A voucher (MARE 572) is deposited in the Herbarium of Faculty of Pharmacy, Univ. of Marmara (Istanbul). Dried and powdered aerial parts of *A. sintenisii* (1.37 kg) was extracted with Et_2O–petrol (1:2) and the extract was treated with MeOH to remove long chain saturated hydrocarbons, the residue was separated by CC (silica gel) the fractions were further separated by prep. TLC. Thus stigmasterol (60 mg), α-amyrin (21 mg), salvigenin (7.3 mg), 6-hydroxyluteolin 6,7,3′4′-tetramethyl ether (15 mg) and sintenin (**1**) (6 mg) were obtained.

Sintenin, 3β,9α-diacetoxycostunolide (**1**): Amorphous, colourless compound. IR $\nu_{\max}^{\mathrm{CHCl_3}}$ cm^{-1} 1760 (γ-lactone), 1720, 1240 (–OAc), 1660 C=C), 1450, 1375, 950, ^{1}H NMR given in Table 1. MS, 70 eV (probe) m/z (rel. int.): 350 $[M]^+$ ($C_{19}H_{26}O_6$) (0.6), 291 $[M-OAc]^+$ (100), 231 $[M-HOAc-OAc]^+$ (60), 203 $[231-CO]^+$ (20), 175 $[203-CO]^+$ (40), 91 $[C_7H_7]^+$ (24).

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ACYCLIC DITERPENES FROM *BIFURCARIA BIFURCATA*

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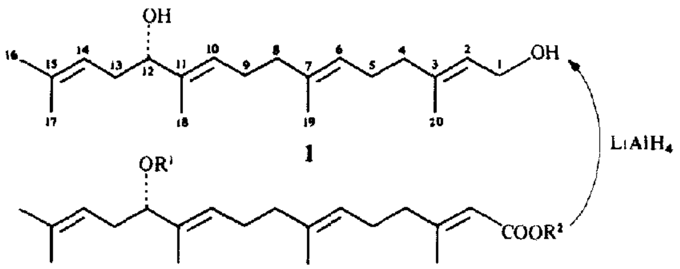
Key Word Index—*Bifurcaria Bifurcata*; Cystoseiraceae; brown alga; marine diterpenoids; (2*E*,6*E*,10*E*)-12(*S*)-hydroxy-3,7,11,15-tetramethyl hexadeca-2,6,10,14-tetraenoic acid; (2*E*,6*E*,10*E*,13*E*)-3,7,11,15-tetramethyl hexadeca-2,6,10,13,15-pentenol.

Abstract—Two new acyclic diterpenes have been isolated from the brown alga *Bifurcaria Bifurcata*. They were characterized by spectral and chemical methods.

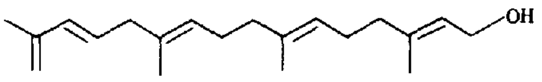
INTRODUCTION

We recently described [1] the main product (**1**) from the brown alga *Bifurcaria Bifurcata* collected along Morocco coasts (Oualidia, 1984). Various acyclic diterpenes have been isolated so far from the family of Cystoseiraceae [2–6], but this re-examination of *Bifurcaria Bifurcata* has led to the isolation of two new acyclic diterpenes, one having an acidic functionality. A few acidic terpenoids have been isolated from marine sources, in particular from marine algae [7].

Silica gel column chromatography of the methanol–chloroform extract of the alga gave a fraction containing **2a** as an impure oil. This fraction was further purified by HPLC to give **2** as an optically active oil. The



2a $R^1 = R^2 = H$
2b $R^1 = Ac$; $R^2 = H$
2c $R^1 = H$; $R^2 = Me$



3

spectral features of **2** (see Table 1 and Experimental) resembled those of related compounds [2–6] and indicated similar structural features. The presence of an hydroxyl functionality was clearly demonstrated by spectral analysis and by acetylation to give **2b**. The ^{1}H and ^{13}C NMR data led unambigously to structure **2a**, with *E* configuration of the double bonds. The presence of an acidic group was confirmed by methylation with diazomethane to yield the corresponding methyl ester **2c** and by reduction with lithium aluminium hydride to give **1** as the end product of the reaction with the same absolute configuration at C-12.

During this investigation, a minor metabolite **3** was also purified by HPLC as an optically inactive oil. This compound showed several spectral features in common with the other acyctic diterpenes, particularly the first three units. The main difference was the presence of a $Me(CH_2{=})C \cdot CH{=}CH$-unit as indicated by the inspection of UV, ^{1}H and ^{13}C NMR data, pointing to structure **3** for the new diterpene.

Table 1. ^{13}C NMR and ^{1}H NMR spectral data for compounds **2a** and **3** in $CDCl_3$ (^{1}H: 360 MHz, TMS as int. stand.; ^{13}C: 50 MHz, TMS as int. stand.)

	2a				**3**		
C/H	^{13}C	^{1}H	*J*(Hz)	C/H	^{13}C	^{1}H	*J*(Hz)
1	171.3 *s*			1	59.0 *t*	4.10 *d*	6.8
2	115.2 *d*	5.66 *s*		2	123.4 *d*	5.37 *t*	6.8
3	162.4 *s*			3	139.1[a] *s*		
4	41.1 *t*	2.17 *m*		4	39.4 *t*	2.00 *m*	
5	26.1 *t*	2.16 *d*	4.5	5	26.5 *t*	2.07 *m*	
6	122.9 *d*	5.06 *t*	4.5	6	123.8 *d*	5.09 *t*	6.8
7	136.0[a] *s*			7	135.0[a] *s*		
8	39.2 *t*	2.00 *m*		8	39.4 *t*	2.00 *m*	
9	25.9 *t*	2.10 *m*		9	26.2 *t*	2.07 *m*	
10	125.9 *d*	5.34 *t*	6.8	10	125.1 *d*	5.13 *t*	6.8
11	136.7[a] *s*			11	133.5[a] *s*		
12	77.3 *d*	3.96 *dd*	6.7	12	42.7 *t*	2.72 *d*	7.2
13	34.2 *t*	2.2 *ddd*	6,7,7	13	128.7 *d*	5.58 *dt*	7.2, 15.6
14	120.2 *d*	5.06 *t*	7	14	133.7 *d*	6.10 *d*	15.6
15	134.4[a] *s*			15	141.9 *s*		
16	25.8 *q*	1.70 *s*		16	114.3 *t*	4.84 *s*	
17	17.9 *q*	1.59 *s*		17	18.5 *q*	1.81 *s*	
18	19.1 *q*	1.60 *s*		18	15.9 *q*	1.57 *s*	
19	16.0 *q*	1.62 *s*		19	15.8 *q*	1.58 *s*	
20	11.6 *q*	2.14 *s*		20	16.1 *q*	1.65 *s*	

[a]: Assignments may be reversed.

EXPERIMENTAL

Freshly collected *Bifurcaria Bifurcata* (Morocco, 1984) was extracted with MeOH–CHCl$_3$. After filtration, the solvents were evapd and the aq. phase was extracted with Et$_2$O. After solvent evapn, 1.95 g extract was obtained and applied to an open column of silica gel. The column was eluted with a solvent gradient from hexane to EtOAc. Compound **2** (0.17% of dry wt of alga) and **3** (0, 02% of dry wt) were eluted with hexane–EtOAc (2:3) and subsequently purified by HPLC (EtOAc–isooctane, 2:3).

(2E,63,10E,)-12(S)-*Hydroxy*-3,7,11,15-*tetramethyl hexadeca*-2,6,10,14-*tetranoic acid* (**2a**). **2a**$[\alpha]_D = +4.19$ (c 8.1, CH$_2$Cl$_2$); IR ν_{max}^{film} cm^{-1}: 3600, 2600, 1685, 1620, 1010; UV $\gamma_{max}^{CHCl_3}$ nm: 281 (8900), 241 (15 500); EIMS (70 eV) *m/z* (rel. int.): 320 (1); HRMS (peak matching): *m/z* 320.2362 calc. for C$_{20}$H$_{32}$O$_3$, 320.2351; ^{1}H and ^{13}C NMR see Table 1. **2b** (Ac$_2$O–pyridine): IR ν_{max}^{film} cm^{-1}: 3600, 1740, 1685, 1620; selected ^{1}H NMR bands (CDCl^3, 360 MHz): δ ppm 2.11 (3H, *s*), 5.17 (1H, *t*, $J = 7$ Hz). **2c** (CH$_2$N$_2$):IR ν_{max}^{film} cm^{-1}: 3600, 1715, 1620; selected ^{1}H NMR band (CDCl3, 360 MHz): δ 3.30 (3H, *s*).

Reduction of **2a** *with* LiAlH$_4$. A cold soln of **2a** (20 mg) in dry Et$_2$O (1 ml) containing LiAlH$_4$ (30 mg) was stirred at 0° for 1.3 hr. Excess reagent was destroyed by slow addition of EtOAc. Addition of saturated MgSO$_4$ soln and extraction with Et$_2$O yielded 15 mg of an oil. After purification by HPLC (EtOAc–isooctane, 2:3), the corresponding reduction product (20 mg) was found to be identical to **1** by comparison of spectral data and with the same absolute configuration of C-12 (Horeau-determination: see ref. [1] for details).

(2E,6E,10E,13E)-3,7,11,15-*tetramethyl hexadeca*-2,6,10,13,15-*pentenol* (**3**). IR ν_{max}^{film} cm^{-1}: 3320, 1665, 1645, 1610; UV $\gamma_{max}^{CHCl_3}$ nm: 281 (8000), 241 (14 000); ^{1}H and ^{13}C NMR see Table 1; EIMS (70 eV) *m/z* (rel. int.): 288 (2); HRMS (peak matching). *m/z* 288. 2465 calc. for C$_{20}$H$_{32}$O, 288.2453.

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