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LINEAR DITERPENOIDS FROM *CYSTOSEIRA BALEARICA*

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Key Word Index—*Cystoseira balearica*; Phaeophyta; Cystoseiraceae; diterpenoids; eleganonal [(2*E*, 6*E*, 10*E*, 14*E*)-13-oxo3,7,11,15-tetramethylpentadeca-2,6,10,14-tetraenal]; *iso*-eleganonal [(2*Z*, 6*E*, 10*E*, 14*E*)-13-oxo-3,7,11,15-tetramethylpentadeca-2,6,10,14-tetraenal].

Abstract—From the brown alga *Cystoseira balearica* we have isolated, in addition to previously reported acyclic diterpenoids, two new compounds (eleganonal and *iso*-eleganonal). Their structures have been elucidated by spectral analysis and chemical correlation.

INTRODUCTION

In the course of our continuing phytochemical investigation of the marine genus *Cystoseira* [1] we recently became aware that an alga previously classified as *C. balearica* (which had yielded two polycyclic meroterpenoids, balearone [2] and cystoketal [3]) is instead a local form of *C. stricta*, a widespread Mediterranean seaweed. An authentic specimen of *C. balearica* Sauv., collected near Trapani, Sicily, revealed a totally different chemical composition, which we wish to report in the present paper.

METHODS AND RESULTS

Seven acyclic diterpenoids have been isolated and characterized from *C. balearica*; five have been positively identified, by comparison with reference samples, with known compounds, namely geranylgeraniol [4] and its acetate [5], eleganolone [6, 7] and its acetate [5], and crinitol [8].

A sixth metabolite (**1**), that we propose to call eleganonal, is an optically inactive oil, molecular formula $C_{20}H_{30}O_2$. Its ^{13}C NMR spectrum (Table 1) indicated the presence of four trisubstituted olefin bonds, five vinyl methyls and two conjugated carbonyls. From the ^1H NMR spectrum (Table 2) it was apparent that one of the carbonyls is embodied in a formyl group (δ9.98), which was established by double resonance to be adjacent to an olefin proton, which was in turn allylically coupled with the methyl resonating at δ2.17. On the other hand, the characteristic chemical shifts of 2H-12 (δ3.12) and H-14 (δ6.11) identified the two groups α to the second carbonyl. This and the long-range couplings of H-10 and 2H-12 with 3H-18, and of H-14 with both 3H-16 and 3H-20, as well as the vicinal coupling of H-10 with 2H-9, defined the structure of the two terminal isoprene units. These data suggest the novel metabolite to be an oxidation product of a known hydroxyketone, eleganolone (**3**), in which the primary alcohol group is replaced by an aldehyde function. This inference was supported by the comparison of both ^1H and ^{13}C NMR spectra of the novel metabolite with those of **3**, and definitely proved by sodium borohydride reduction of **1**, which gave a product indistinguishable from eleganolone.

The last metabolite isolated, *iso*-eleganonal **2**, is an isomer of **1**, to which it resembles closely in the mass fragmentation (see Experimental). Also the ^1H NMR

Table 1. ^{13}C NMR of compounds **1–3** (75.5 MHz, $CDCl_3$, TMS as int. standard)*

Position	1	2	3†
C-1	191.2 *d*	190.2 *d*	59.4 *t*
C-2	127.4 *d*[a]	128.8 *d*[a]	124.4 *d*
C-3	163.6 *s*	163.6 *s*	139.4 *s*
C-4	40.0 *t*[b]	32.6 *t*	39.7 *t*
C-5	25.5 *t*[c]	26.7 *t*[b]	26.4 *t*
C-6	122.6 *d*[d]	122.6 *d*	124.1 *d*
C-7	136.2 *s*	136.2 *s*	135.3 *s*
C-8	39.2 *t*[b]	40.0 *t*	39.4 *t*
C-9	26.6 *t*[c]	26.5 *t*[b]	26.9 *t*
C-10	122.8 *d*[d]	122.6 *d*	123.2 *d*
C-11	129.8 *s*	129.8 *s*	129.8 *s*
C-12	55.4 *t*	55.4 *t*	55.5 *t*
C-13	199.4 *s*	199.4 *s*	199.9 *s*
C-14	128.8 *d*[a]	127.4 *d*[a]	129.7 *d*
C-15	155.4 *s*	155.4 *s*	155.8 *s*
C-16	27.6 *q*	27.6 *q*	27.7 *q*
C-17	17.7 *q*	25.2 *q*	16.0 *q*
C-18	16.0 *q*[e]	16.0 *q*[c]	16.0 *q*
C-19	16.4 *q*[e]	17.7 *q*[c]	16.4 *q*
C-20	20.8 *q*	20.8 *q*	20.7 *q*

*Multiplicities were obtained by off-resonance decoupling experiments; resonance values of C-16 and C-20 as reported in ref. [7] have been interchanged on the basis of specific proton–carbon decoupling experiments.

†Added for comparison.

[a–e]Values with identical superscripts within a column can be interchanged.

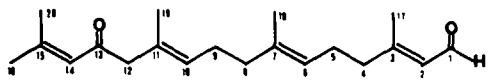
Table 2. ^{1}H NMR of compounds 1–3 (300 MHz, $CDCl_3$, TMS as int. standard)*

Position	1	2	3†
H-1	9.98 *d* (8)	9.88 *d* (8)	4.15 *d* (6.5)
H-2	5.86 *d* (8)	8.86 *d* (8)	5.40 *d* (6.5)
H-4	*ca* 2‡	2.57 *t* (7.5)	2.04
H-5	*ca* 2‡	*ca* 2‡	2.04
H-6	5.09 *t* (6)	5.08 *t* (6)	5.10 *t* (6.5)
H-8	*ca* 2‡	*ca* 2‡	2.04
H-9	*ca* 2‡	*ca* 2‡	2.04
H-10	5.22 *t* (6.5)	5.22 *t* (6.5)	5.21 *t* (6.5)
H-12	3.02 *s*	3.02 *s*	3.03 *s*
H-14	6.11 *br s*	6.11 *br s*	6.10 *s*
H-16	1.86 *s*	1.86 *s*	1.87 *s*
H-17	2.17 *s*	1.96 *s*	1.66 *s*
H-18	1.60 *s*	1.60 *s*	1.60 *s*
H-19	1.60 *s*	1.60 *s*	1.60 *s*
H-20	2.17 *s*	2.12 *s*	2.12 *s*

*Chemical shifts are δ values; coupling constants (J in parentheses) are given in Hz; assignments were confirmed by decoupling.

†Added for comparison (see refs [4, 5]).

‡Overlapped with other signals.



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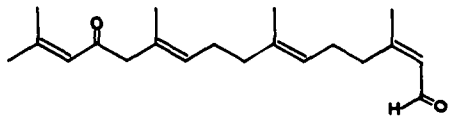

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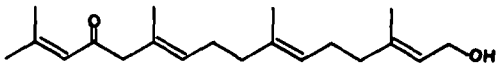

3

spectrum of 2 is very similar to that of 1 (Table 2), a single characteristic difference being the downfield shift of the methylene protons at C-4 from *ca* δ2 in 1 to δ2.57 in 2 and the concurrent upfield shift of the vinyl methyl at C-3 from δ2.17 in 1 to δ1.96 in 2. This is consistent with a change of the geometry of the C-2 double bond from *E* in 1 to *Z* in 2. As expected, the ^{13}C NMR spectrum of 2 differs from that of 1 essentially in the chemical shifts of C-4 (32.6 ppm in 2 as compared to 40.0 ppm in 1) and C-20 (25.2 against 17.7). Aldehyde 2 is a labile compound, that at room temperature slowly converts into the more stable 1.

EXPERIMENTAL

General. Chemical shifts of NMR data are given in δ values (ppm) with TMS as int. standard. MS were performed with a direct inlet system at 70 eV. HRMS were recorded on a Kratos MS-50S spectrometer. Preparative liquid chromatography (PLC) was carried out on a Jobin-Yvon MiniPrep LC instrument. Known compounds were identified by TLC, MS and ^{1}H NMR comparison with authentic samples.

Plant material. Cystoseira balearica Sauv. (voucher specimen deposited in the Herbarium of the Department of Botany, Palermo, Italy) was collected at about 3 m depth in June 1986 near Trapani (Isola del Porcello), Sicily.

Extraction and isolation of constituents. Ground air-dried thalli of *C. balearica* (200 g) were extracted × 3 with CH_2Cl_2 at room temp. with continuous stirring. Bulked extracts were evaporated by red. pres. to a viscous residue (2.5 g) which was chromatographed on a silica gel column (50 × 2 cm, increasing concentrations of Et_2O in hexane as the eluent). Fractions of 20 ml were collected and those exhibiting similar TLC profiles combined. The less polar fractions gave geranylgeraniol acetate (fractions 5–6, 43 mg) and eleganolone acetate (fractions 14–15, 77 mg), while the more polar ones yielded eleganolone (fractions 28–31, 710 mg) and crinitol (fractions 39–41, 35 mg).

Further fractionation by PLC (LiChroprep Si-60; C_6H_{14}–CH_2Cl_2 3:7 followed by C_6H_{14}–AcOEt 10:1 as the eluents) of the fractions of intermediate polarity (fractions 18–22) gave 1 (125 mg), 2 (119 mg) and geranylgeraniol (377 mg).

Eleganonal 1. Oil; IR ν_{max}^{film} 1690, 1680, 1625 cm^{-1}; UV λ_{max}^{EtOH} nm (ε): 239 (26500); HRMS [M$^+$] 302.2242 (calc. for $C_{20}H_{30}O_2$ 302.2245); MS *m/z* (rel. int.): 302 (8), 284 (1), 219 (20), 204 (6), 202 (4), 187 (5), 186 (7), 159 (3), 152 (22), 149 (9), 138 (15), 135 (9), 123 (26), 121 (11), 108 (12), 95 (11), 84 (41), 83 (100), 68 (13), 55 (72), 53 (14), 43 (8), 41 (20); ^{1}H and ^{13}C NMR see Tables 1 and 2.

Iso-*eleganonal* 2. Oil; IR ν_{max}^{film} 1690, 1680, 1625 cm^{-1}; UV λ_{max}^{EtOH} nm (ε): 239 (25700); HRMS [M$^+$] 302.2248 (calc. for $C_{20}H_{30}O_2$ 302.2245); MS *m/z* (rel. int.): 302 (6), 284 (3), 276 (9), 219 (7), 204 (6), 201 (5), 187 (7), 186 (13), 159 (5), 151 (32), 149 (9), 138 (25), 134 (15), 123 (35), 123 (35), 121 (10), 108 (15), 95 (18), 84 (61), 83 (100), 66 (23), 55 (89), 43 (44), 41 (32); ^{1}H and ^{13}C NMR see Tables 1 and 2.

$NaBH_4$ reduction of 1 *to produce* 3. $NaBH_4$ (3 mg) was added to a soln of 1 (50 mg) in EtOH (2 ml) and the mixture was stirred at 0° per 10 min. After addition of H_2O, the organic material was extracted with Et_2O. The combined extracts were dried (Na_2SO_4) and evaporated *in vacuo* to yield 40 mg of an oil, homogeneous by TLC, whose physical properties (IR, UV, MS, ^{1}H and ^{13}C NMR) were identical with those of 3.

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LABDANE DITERPENES FROM *BRICKELLIA GLOMERATA**

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Key Word Index—*Brickellia glomerata*; Compositae; Eupatorieae; labdane type diterpenes; *enantio*-oliveric acid; 12-oxolambertianic acid; demethyl pinusolide; lambertianic acid.

Abstract—The leaves of *B. glomerata* afforded three new labdane diterpenes. Their structures and stereochemistry were established by spectroscopic methods and chemical transformations.

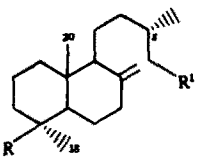
INTRODUCTION

In continuation of our chemical studies of members of the genus *Brickellia* (Eupatorieae) [1–4], we have undertaken the study of *B. glomerata* and have isolated in addition to the known lambertianic acid, three new labdane type diterpenes whose structures were established as enantio-oliveric acid (**1a**), 12-oxolambertianic acid (**2b**) and demethyl pinusolide (**3a**).

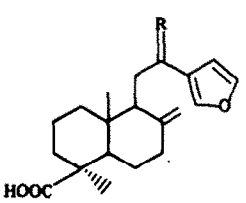
RESULTS AND DISCUSSION

Compound **1a** was the major constituent of the leaves of *B. glomerata*, $C_{20}H_{32}O_4$, $[M]^+$ at m/z 336, was isolated as a gum. The presence in **1a** of carboxylic acid group(s) were shown by IR absorptions at 3500–2380 and 1695 cm^{-1}, and by the formation of the dimethyl ester (**1b**) upon treatment with excess of diazomethane. It also contained an exocyclic methylene group (IR absorptions at 1640 and 890 cm^{-1}). The ^1H NMR spectrum of **1a** showed signals due to two tertiary methyl groups (δ0.55 and 1.27) and one secondary methyl group (δ0.95, *d*, J = 6.5 Hz). The presence of the exocyclic methylene group was confirmed by absorptions at δ4.47 and 4.80 (*br s*, 1H each).

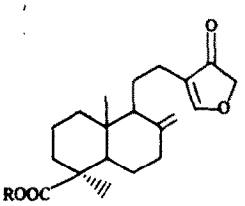
The hindered nature of one of the carboxylic groups in **1a** followed from the formation of the mono ester **1c**, obtained by Fischer esterification, while alkaline hydrolysis of **1b** gave the isomeric mono-ester **1d**. Additional proof for the axial configuration of the carboxyl group at C-4 was provided by reduction of **1a** with lithium aluminium hydride to the diol **1e**. The ^1H NMR spectrum of **1e**, showed two doublets at δ3.36 and 3.75 (AB system) characteristic of an axial hydroxy methylene group at C-4 [5–6].



1a R = R^1 = COOH
1b R = R^1 = COOMe
1c R = COOH, R^1 = COOMe
1d R = COOMe, R^1 = COOH
1e R = R^1 = CH_2OH



2a R = H_2
2b R = O



3a R = H
3b R = Me

*Contribution No. 844 from Instituto de Química, UNAM.