Journal of Natural Products
Vol. 47, No. 6, pp. 947-952, Nov-Dec 1984

CYSTOKETAL, A NEW METABOLITE FROM THE BROWN ALGA *CYSTOSEIRA BALEARICA*

VINCENZO AMICO, FRANCESCA CUNSOLO, GIOVANNA ORIENTE, MARIO PIATTELLI,

Istituto Dipartimentale di Chimica dell'Università di Catania, V. le A. Doria 6, 95125 Catania, Italy

and GIUSEPPE RUBERTO

Istituto del CNR per lo studio delle sostanze naturali di interesse alimentare e chimico-farmaceutico, V.le A. Doria 6, Catania, Italy

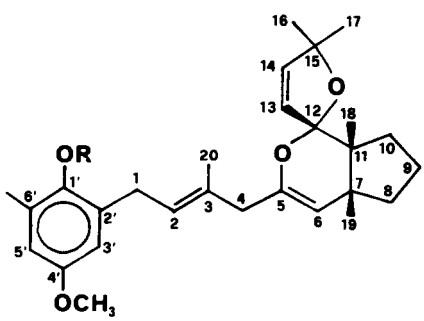
ABSTRACT.—A new metabolite of mixed biogenesis, cystoketal **1**, has been isolated from the brown alga *Cystoseira balearica* and its structure, including relative stereochemistry, determined by spectral methods.

In the course of our continued studies of brown algae of the family Cystoseiraceae, we have reported the structure of many tetraprenyl-hydroquinol derivatives (1-6). The present investigation with *Cystoseira balearica* Sauv. describes the isolation and structure elucidation of a new member of this family of metabolites, cystoketal **1**.

Compound **1** was isolated from the $CHCl_3$ extract of the alga as an optically active oil, $[\alpha]D = 11.5°$. Its elemental composition was established as $C_{28}H_{38}O_4$ by hrms. The ir spectrum showed an intense hydroxyl absorption at 3470 cm^{-1} and further illustrated the absence of a carbonyl function. Uv absorption at 220 ($\epsilon = 10500$) and 287 nm ($\epsilon = 3150$) were indicative for a hydroquinol chromophore. Acetylation of **1** afforded a monoacetate **2**, $C_{30}H_{40}O_5$, in the ir spectrum of which the OH absorption had disappeared. Hence, three out of the four oxygen atoms present in **1** are of the ether type. One of them is embodied in an aromatic methoxyl (^{13}C-nmr: 53.6 ppm, q; ^{1}H-nmr: δ 3.75, s), while the remaining two are adjacent to the same carbon, as the ^{13}C-nmr spectrum of **1** (Table 1) contains in the low-field region between 109 and 153.3 ppm an odd number of lines, namely 13.

The mass spectrum of **1** displayed diagnostically important peaks at *m/z* 150 (base), 151 (20%), 189 (11%), 191 (22%), and 205 (18%), which were considered, based on the known fragmentation of related compounds (7), strongly indicative of partial structure **A** from which they can originate according to Scheme 1.

This structural hypothesis was supported by the 500-MHz ^{1}H-nmr spectrum, which contained, in addition to the methoxyl resonance, a D_2O-exchangeable 1H signal at δ 4.88 (phenolic OH), an AB system (δ 6.58 and 6.51, $J = 3$ Hz) assignable to two *meta*-coupled aromatic protons, a methyl on benzene ring at δ 2.21, and an ABX



1 R=H
2 R=$COCH_3$

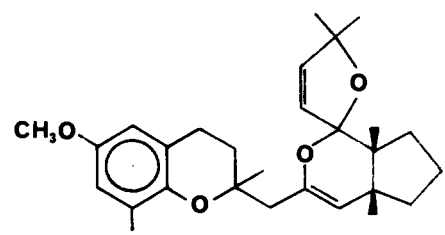


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TABLE 1. ^{13}C-nmr Assignments for Cystoketal (**1**) and Chromane (**3**)[a]

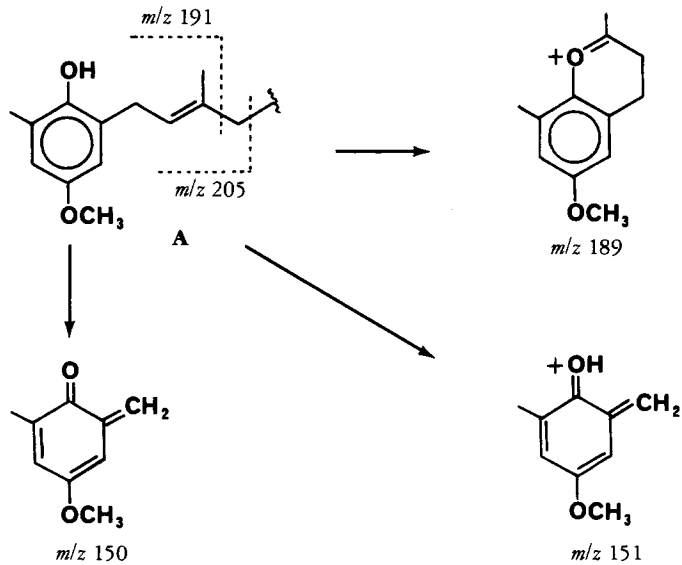
Carbon Atom	Compounds		Carbon Atom	Compounds	
	1	**3**		**1**	**3**
C-1′	146.5*s*[b]	146.3*s*[b]	C-9	20.4*t*	20.4*t*
C-2′	136.0*s*	121.1*s*	C-10	36.1*t*	36.3*t*
C-3′	114.0*d*[c]	114.9*d*[c]	C-11	46.3*s*	46.1*s*
C-4′	153.3*s*	152.5*s*	C-12	115.0*s*	115.3*s*
C-5′	113.0*d*[c]	111.2*d*[c]	C-13	140.0*d*	140.2*d*
C-6′	127.0*s*[d]	127.4*s*	C-14	126.8*d*	126.9*d*
C-1	30.8*t*	22.8*t*	C-15	88.0*s*	88.2*s*
C-2	123.8*d*	31.3/31.5*t*[g]	C-16	26.3*q*[e]	26.4*q*[d]
C-3	126.0*s*[d]	75.8*s*	C-17	28.7*q*[e]	28.9*q*[d]
C-4	45.1*t*	44.2/44.5*t*[g]	C-18	20.2*q*[f]	20.3*q*[e]
C-5	147.1*s*[b]	145.8*s*[b]	C-19	22.7*q*[f]	22.8*q*[e]
C-6	109.0*d*	111.2*d*	C-20	15.6*q*	25.0*q*
C-7	43.2*s*	43.1*s*	C6′-Me	16.2*q*	16.3*q*
C-8	40.5*t*	40.7*t*	C4′-OMe	53.6*q*	55.7*q*

[a]Recorded in $CDCl_3$ at 20.1 MHz with TMS as internal standard; values are given in ppm. Multiplicities were obtained by off-resonance decoupling with the aid of INEPT sequence. Assignments are based on signal multiplicities and comparison with model compounds (1-6).

[b-f]Assignments may warrant changing.

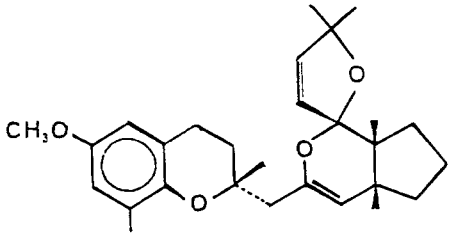
[g]Doubleting is due to stereoisomerism about the chiral center at C-3.

pattern [δ 5.38 (1H, t, J=7 Hz, X-part of an ABX) and an AB-part of an ABX centered at δ 3.43 (1H, dd, J=16, 7.7 Hz) and 3.24 (1H, dd, J=16, 6.3 Hz)] associated with the three-spin system of the benzylic methylene and the adjacent olefinic proton. The 3-CH_3 appeared as a singlet (δ 1.78) allylically coupled with the vinyl proton at δ 5.38, and the C-4 methylene as an AB system (δ 2.74 and 2.69, each 1H, J=15 Hz). The location of the substituents around the benzene nucleus was confirmed by nuclear Overhauser enhancement difference spectroscopy (nOeds) experiments: the signals of the aromatic protons at δ 6.58 and 6.52 were enhanced by irradiation of the aromatic methyl or, respectively, the benzylic methylene (~ 15% in both cases).

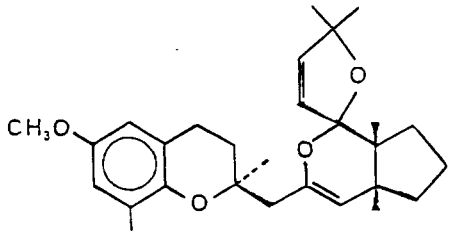


SCHEME 1.

The ^{13}C-nmr spectrum of **1** showed all the appropriate resonances for partial structure **A**, which was definitely proved by cyclization of **1** to an isomeric mixture of chromanes **3a** and **3b**. The ^{13}C-nmr spectrum of this mixture when compared with



3a

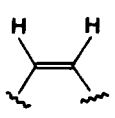


3b

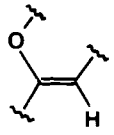
that of **1** (Table 1) displayed all the differences expected from examination of a model compound (3): (a) two fewer olefinic resonances in the low-field region; (b) two newly generated lines in the sp^3 region; (c) an upfield shift of the resonance associated with carbons C-2′ and C-1; (d) a downfield shift of the resonance associated with 4-CH_3. The observed doubleting of the signals pertaining to the methylene carbons C-2 (31.3 and 31.5 ppm, t) and C-4 (44.2 and 44.5 ppm, t) is obviously caused by stereoisomerism about the chiral center at C-3. The most significant variations in the proton spectrum of the chromane mixture in comparison with that of **1** are the disappearance of the resonance of the OH, the replacement of the ABX pattern pertaining to the protons at C-1 and C-2 with show two 2H multiplets at δ 2.71 (C-1) and 1.7 (C-2), and the shift of the signal associated with the C-4 methylene from a doubly-allylic to an allylic position (δ 2.26). Moreover, the resonance of the 3-CH_3 suffered an upfield shift, with concurrent doubleting (δ1.37 and 1.38) resulting from stereoisomerism at C-3.

At this stage, taking into account that the elemental formula of **1** implies ten degrees of unsaturation, it could be inferred that the rest of the molecule must incorporate two double bonds and three rings. One of the double bonds in question is disubstituted and appears in the proton spectrum as an AB system centered at δ 6.02 and 5.65 (each 1H, d, J=5.5 Hz); the small value of the coupling constant indicates that it is incorporated in a five-membered ring. The other one is trisubstituted and the shielding of the relevant olefinic proton (δ 4.35) discloses the presence of an oxygen atom in β-position. The spectrum also contained two methyl singlets on an oxygen-bearing carbon at δ 1.32 and 1.28, and two methyls on quaternary carbons at δ 1.15 and 0.89. Complex signals (centered at δ 1.99, 1.69, 1.57, and 1.37) account for six protons altogether and, on the basis of the ^{13}C-nmr spectrum, must belong to three methylenes. Although a complete analysis of these signals could not be accomplished owing to the complexity of the six-spin system, homonuclear decoupling experiments gave evidence that the methylenes in question are contiguous and not coupled with the rest of the hydrogens.

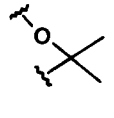
The above ^{1}H-nmr data, considered in comparison with model compounds (4,5), secured the following structural elements (**B-E**), for which there is also evidence in the ^{13}C-nmr spectrum (**B**, 140.0 d, 126.8 d; **C**, 147.1 s, 109.0 d; **D**, 88.0 s, 28.7 q, 26.3 q; **E**, 46.3 s, 43.2 s, 40.5 t, 22.7 q, 20.4 t, 20.2 q).



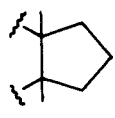
B



C

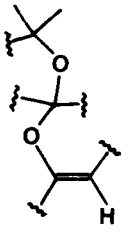


D

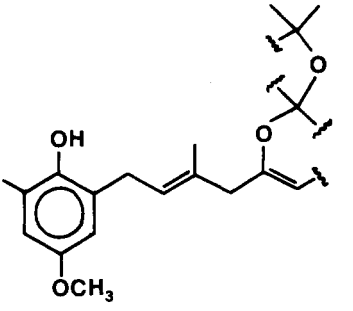


E

The only atom in the molecule of **1** not accounted for by part structures **A-E** is the low-field resonanting, quaternary sp^3-carbon (115.0 s), which evidently connects moieties **C** and **D** through their oxygen atoms as in subunit **F**. Because the C-4 methylene appears as an AB system, it must be isolated and, on account of its chemical shift, in a doubly-allylic position. Moiety **A** must be linked, therefore, to the non-hydrogen-bearing sp^2-carbon of subunit **F** and this allows partial structure **G** to be derived.



F



G

To complete the structure, only part **B** and **E** are to be added in such a way to form a tricyclic system in which the disubstituted double bond must be implied in a five-membered ring. This unavoidably leads to structure **1** (devoid of stereochemistry) for the new algal metabolite and **3** for the corresponding chromane. The *trans* nature of the C-2 double bond in **1** was indicated by the chemical shift of the 3-CH_3 (15.6 ppm) in the ^{13}C-nmr spectrum (8), while the relative stereochemistry of the chiral centers at C-7, C-11, and C-12 depicted in structure **1** was deduced from nOeds data (table 2), which require the angular methyls and the olefinic proton at C-13 to be on the same face of the molecule. In addition, these results indicated a preferred conformation in which 3-CH_3 and H-6 are within nOe distance (less than 3.5 Å).

TABLE 2. Results of nOeds Experiments on Cystoketal (**1**)

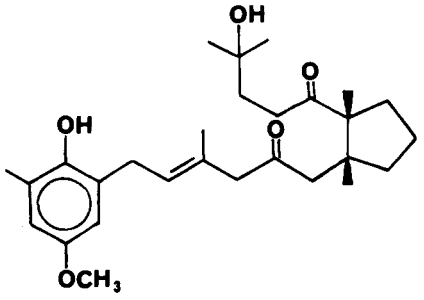
Signal irradiated	Signal enhanced	% enhancement
Me-7	Me-11 (δ 0.89 s)	19
	$H\text{-}10_a$ (δ 1.37 m)[a]	5
	$H\text{-}9_a$ (δ 1.69 m[a]	7
	H-13 (δ5.65 d)	10
Me-11	Me-7 (δ 1.15 s)	20
	$H\text{-}9_a$ (δ 1.69 m)[a]	9
	Me-3 (δ 1.76 s)	7
	H-6 (δ 4.35 s)	11

[a]Protons $H\text{-}9_a$ and $H\text{-}10_a$ and the angular methyls are on the same face of the molecule.

Finally, it is to be noted that chromane **3** has been isolated from *C. balearica*, along with **1**, as an epimeric mixture at C-3, a fact that indicates it to be an artifact of the extraction process or perhaps a compound formed in the living alga through a reaction not controlled enzymically.

Compound **1** can be supposed to derive biogenetically from an intermediate such as **4**, recently isolated from the congener species *Cystoseira algeriensis* (5), through enoliza-

tion of the C-5 carbonyl followed by nucleophilic attack of the enolic hydroxyl to the C-12 carbonyl and further manipulations.



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EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mass spectra were performed with direct injection system at 70 eV on an AEI MS 902 instrument. Ir spectra were run in CCl_4 solutions on a Perkin-Elmer model 684 spectrophotometer. Uv spectra were obtained on a Perkin-Elmer 330 instrument. ^{1}H-nmr spectra were measured at 500, 250, and 80 MHz with Bruker WM-500, WM-250, and WP-80 instruments, respectively. ^{1}H-nOeds were run at 500 MHz in degassed $CDCl_3$ solution. ^{13}C-nmr spectra were obtained at 20.1 MHz on a Bruker WP-80 instrument. Chemical shifts are quoted in ppm (δ) relative to TMS. Optical rotations were determined with Perkin-Elmer 141 polarimeter. Tlc was carried out using glass precoated silica gel F_{254} plates (Merck). Spots were detected by spraying with 1% solution of $Ce(SO_4)_2$ in 2N H_2SO_4. Preparative liquid chromatography (plc) was carried out on a Jobin-Yvon Miniprep LC instrument.

PLANT MATERIAL.—*C. balearica* was collected at about 1 m depth in April 1982, near Portopalo, Sicily. A voucher specimen was deposited in the Herbarium of the Institute of Botany, Catania, Italy.

EXTRACTION AND PURIFICATION.—Shade-dried and ground alga (1.7 kg) was extracted three times with $CHCl_3$ at room temperature under continuous stirring. Evaporation of the pooled extracts gave 35 g of oily residue that was applied to an open column (4×120 cm) of Si gel and eluted with increasing concentrations of Et_2O in hexane. Fractions of 200 ml each were collected, and those exhibiting similar profiles were combined.

Fractions 20-32 were pooled and subjected to successive plc (LiChroprep Si-60, 25-40 μm) using C_6H_{12}-isopropylether (90:10) and C_6H_6-hexane (70:30) in that order, thus giving **1** (290 mg, 0.017% dry weight of the alga). Compound **1**, oily, $[\alpha]^{20}$ (λ): +11.5° (589), +12.7° (578), +15° (546) (*c*=1 in EtOH); ir ν max (CCl_4) 3470, 1685, 1610 cm^{-1}; uv λ max (EtOH) 220 (ε=10500), 287 nm (ε=3150); hrms M^+ 438.2765 (calc. for $C_{28}H_{38}O_4$ 438.2769); ms *m/z* (%): 438 (20), 420 (3), 289 (7), 288 (33), 223 (38), 205 (18), 191 (22), 189 (11), 167 (11), 151 (20), 150 (base), 149 (93), 137 (20), 135 (16), 109 (13), 105 (13), 104 (16), 95 (13), 91 (13), 69 (13), 57 (60), 56 (16), 55 (16), 43 (13), 41 (27).

Fractions 6-15 were evaporated to give an oily residue that was subjected to plc (LiChroprep-60, 25-40 μm; hexane-Et_2O, 98:2) to give **3** (170 mg, 0.010% dry weight). Compound **3** had ir ν max (CCl_4) 1680, 1605 cm^{-1}; uv λ max (EtOH) 220 (ε=9500, 295 (ε=3200); hrms M^+ 438.2774 (calcd. for $C_{28}H_{38}O_4$ 438.2769); ms *m/z* (%) 438 (59), 420 (13), 288 (59), 270 (19), 191 (78), 189 (27), 175 (16), 150 (base), 137 (32), 135 (35), 123 (19), 121 (25), 109 (27), 95 (32), 91 (40), 81 (24), 69 (16), 55 (25), 43 (39), 41 (38); ^{1}H-nmr (250 MHz, $CDCl_3$, δ-scale) 6.54 and 6.41 (AB system, each 1H, d, *J*=3 Hz, H-5′ and H-3′), 6.02 and 5.57 (AB system, each 1H, d, *J*=5.5 Hz; H-14 and H-13), 4.33 (1H, s; H-6), 3.71 (3H, s; -OCH_3), 2.71 (2H, t, *J*=7.5 Hz; H-1), 2.26 (2H, s; H-4), 2.13 (3H, s; 6′-CH_3), 1.7 (2H, overlapped, H-2), 1.37 and 1.38 (3H altogether, s; H-20), 1.33 (3H, s; H-17), 1.30 (3H, s; H-16, 1.15 (3H, s; H-19), 0.87 (3H, s; H-18), 1.9, 1.6, 1.5, 1.4 (complex signals, 6H altogether; H-8, H-9 and H-10).

ACETYLATION OF **1** TO GIVE **2**.—Compound **1** was acetylated overnight at room temperature with Ac_2O-pyridine. Purification by plc (LiChroprep Si-60, 25-40 μm; C_6H_6-hexane, 55:45) gave pure **2**; ir ν max (CCl_4) 1730 cm^{-1}; hrms M^+ 480.2872 (calcd. for $C_{30}H_{40}O_5$ 480.2875); ms *m/z* 480 [M^+], 438 [M^+-CH_3CO]; ^{1}H-nmr (80 MHz, $CDCl_3$, TMS) δ 2.12 (3H, s, CH_3COOAr).

CYCLIZATION OF **1** TO GIVE **3a** AND **3b**.—Compound **1** (30 mg) in C_6H_6 (2 ml) was refluxed for 10 h in the presence of Florisil (300 mg). After purification by plc (LiChroprep Si-60, 25-40 μm; hexane-

Et_2O, 98:2) a mixture of **3a** and **3b** was obtained, identical in all respects to the compound isolated from the alga.

ACKNOWLEDGMENTS

The work was financially supported by the Consiglio Nazionale delle Ricerche (Rome) under the scheme "Progetto Finalizzato per la Chimica Fine e Secondaria."

We wish to thank Dr. L. Mayol (Department of Natural Substances, University of Naples) and Dr. E. Trivellone (Istituto per lo studio di molecole di interesse biologico del CNR, Naples) for measurements of ^{1}H-nmr spectra on 250 and 500 MHz, respectively.

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Received 15 March 1984