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# METHOXYCYSTOKETAL QUINONE: NATURAL COMPOUND FROM BIOACTIVE DIETHYL ETHER EXTRACT OF BROWN SEAWEED *CYSTOSEIRA TAMARISCIFOLIA*

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**Abstract**

A meroditerpenoid compound has been isolated from the bioactive fraction of diethyl ether extract of brown seaweed *Cystoseira tamariscifolia* and characterized as Methoxycystoketal quinone. Their structure has been elucidated on the basis of in-depth spectroscopic analyzes : $^{1}H$ and $^{13}C$-NMR, MS, UV and IR. The antibacterial activities of the molecule isolated against *Cutibacterium acnes* (ATCC 6919) and *Staphylococcus epidermidis* (ATCC14990) were tested. The results showed that this compound derived from cystoketal possessed remarkable activity.

**Keywords**: *Meroditerpenoid, Cystoseira tamariscifolia, Brown seaweed, Antibacterial activity.*

## Introduction

Several species of the brown algal genus *Cystoseira* (Cystoseiraceae, Pheophyta) are widespread in the Atlantic and Mediterranean sea, but very few of them have been reported to contain biologically active compounds [1-4]. Many tetraprenyltoluquinols have been isolated, either with a regular diterpenoid moiety, such as: cystoketal, bifurcarenone, balearone, amentol, strictaketal, amentadione, amentaepoxide, … [5-7] or with a rearranged one, such as: neobalearone and 2-epineobalearone [8-10].

To our knowledge, one data has been reported on the metabolites of *Cystoseira tamariscifolia* collected in the western part of the Moroccan ocean, it is Methoxybifurcarenone which exhibited significant microbial activities [11]. In this article, we describe the isolation and elucidation of the structure of a natural meroditerpene C from this alga which was present with compounds A and B previously studied in the lipid extracts of other brown algae of the genus Cystoseiraceae. Their quantitative determination is also reported with antibacterial activity against two strains responsible for acne such as: *Cutibacterium acnes* (ATCC 6919) and *Staphylococcus epidermidis* (ATCC14990).

## Materiel and methods

### Plant material

*Cystoseira tamariscifolia* was collected in February 2020 at Dar Bouaaza (Casablanca, Morocco) for isolation of compound C. A voucher specimen of this seaweed is deposited in the Herbarium of EST-Khenifra, University Sultan My Slimane.

### Extraction and purification

The shade-dried material of brown seaweed *Cystoseira tamariscifolia* (2 Kg) was ground and maceration extract with solvents of increasing polarity: hexane, diethyl ether, chloroform and water. After filtration and evaporation of solvent, 18 g of a crud diethyl ether extract were obtained and subject to column chromatography on silica gel eluted with a solvent gradient from hexane to ethyl acetate. The compound C was eluted with hexane – diethyl ether (3:1). It was subsenquently purified by semi-prep normal phase HPLC (Ethyl acetate – Isooctane) to give 422 mg C.

### Experimental and analysis techniques

Electronic spectra were taken on a Shimadzu spectrophotometer in ethanol (UV-265FS, Shimadzu, Kyoto, Japan). NMR spectra were recorded in $CDCl_3$ at 300 MHz using Varian Unity 600 instrument (Varian Inc., Palo Alto, CA, USA). The chemical shifts (δ) are reported in ppm and the coupling constants in Hz. HREIMS and EI measurements were carried out on a Fisons VG Autospec (Fisons, Manchester, UK). HPLC was carried out on a Hewlett–Packard HP 1090 instrument (Hewlett–Packard, Palo Alto, CA, USA) operating at 254 nm equipped with a normal column. All reagents and solvents were used as received from commercial sources (Sigma–Aldrich, France).

Compound C. (Methoxycystoketal quinone) Oil; 1H and 13C NMR : Table 1 and2 ; HRMS: $[M]^+$ 438.2406 (Calc. for $C_{27}H_{34}O_5$, 438.2402); EIMS (70eV) m/z (rel.int.) 438 (2), 237 (15), 207 (19), 191 (14), 177 (24), 150 (31), 137 (21), 109 (21), 96 (45), 85 (24), 81(31), 69 (48), 55 (38), 43 (100), 31 (14); IR $\nu_{max}$ (cm-1) : 3020, 3000, 1655, 1650, 1614, 1450, 1370, 1292, 1195, 1106, 1085, 1045,1036, 980, 912, 855, 800; UV $\lambda_{max}$ (nm) : 224 (8522) , 261 (12541).

### Antibacterial activity

The antibacterial test of the compound C was carried out against strains: *Cutibacterium acnes* (ATCC 6919) and *Staphylococcus epidermidis* (ATCC14990) by the microdilution method [12]. The activity tests were carried out in liquid medium in 96-well microplates. The compound is dissolved beforehand in DMSO followed by solubilization in sterile distilled water. Positive growth controls are carried out in the absence of the compound to be tested and a negative control with culture medium in the absence of the target bacteria, are carried out in parallel. The microplates are incubated at 37 ° C. for one day. A measurement of the turbidity of the presence of bacteria by measurement of the absorbance (ABS) at the wavelength 600 nm is carried out. The MIC of compound (minimum inhibitory concentration) are determined from 3 independent repetition.

## Results and discussion

The fractionation of the diethyl ether extract (18g) obtained from an extraction by maceration of the brown seaweed *Cystoseira tamariscifolia* collected from the region of Casablanca (Morocco) was carried out and revealed the presence of a minority compound C (2.11 ‰).

The MS spectrum of Methoxycystoketal quinone how a molecular ion at m/z = 438 compatible with a molecular formula $C_{27}H_{34}O_5$. The infrared spectrum of methoxybifurcarenone showed absorptions for methoxy ($\nu_{OCH3}$= 3020 $cm^{-1}$) and and p-benzoquinone functionalities at 1655, 1650 and 1614 $cm^{-1}$, which was confirmed by UV absorption at 261 nm (12541).

The $^{1}H$ and $^{13}C$ NMR spectral data have been displayed in Table 1 and 2 which gives the information of the chemical shifts of compound C (Methoxycystoketal quinone) with two other molecules similar with their structure such as: compound A (Cystoketal) [13] and compound B (Cystoketal quinone) [14] (Figure 1). A quick read confirms the structure of Methoxycystoketal quinone which has the same values as Cystoketal quinone with a value of OMe-3 ($^{1}H$ NMR: δ 3.52 and $^{13}C$ NMR: 55.8).

The antibacterial activity of compound C (Methoxycystoketal quinone) against two strains responsible for acne: *Cutibacterium acnes* and *Staphylococcus epidermidis* gives values of the minimum inhibitory concentration (MIC) respectively 1.322 mg/ml and 0.985 mg/ml (Table 3). These results confirm the importance of this biomolecule in pharmaceutical and cosmetic preparations for the treatment of dermatological diseases.

## Conclusion

The structure elucidation of a natural metabolite methoxycystoketal quinone isolated from *Cystoseira tamariscifolia* was determined on the basis of spectroscopic analysis techniques such as: NMR, MS, IR and UV. this molecule which has a structure derived from two molecules A (Cystoketal) and B (Cystoketal quinone). The antibacterial activity of this compound against the two bacterial strains of acne shows a new way of valorization *Cystoseira* seaweeds in general and derivatives of cystoketal in particular.

## References


1. Abdel-Raouf, N., Mohamed, H., Mostafa, S., & Ibraheem, I. (2017). Controlling of microbial growth by using Cystoseira barbata extract. Egyptian Journal of Botany, 57(3), 469-477.
2. Abu-Khudir, R., Ismail, G. A., & Diab, T. (2021). Antimicrobial, antioxidant, and anti-tumor activities of Sargassum linearifolium and Cystoseira crinita from Egyptian Mediterranean Coast. Nutrition and cancer, 73(5), 829-844.
3. Bahramzadeh, S., Tabarsa, M., You, S., Li, C., & Bita, S. (2019). Purification, structural analysis and mechanism of murine macrophage cell activation by sulfated polysaccharides from Cystoseira indica. Carbohydrate polymers, 205, 261-270.
4. Ainane, T., Abourriche, A., Kabbaj, M., Elkouali, M., Bennamara, A., Charrouf, M., Lemrani, M. (2014). Biological activities of extracts from seaweed Cystoseira tamariscifolia: Antibacterial activity, antileishmanial activity and cytotoxicity. J. Chem. Pharm. Res, 6, 607-611.
5. Moussa, H., Quezada, E., Viña, D., Riadi, H., & Gil-Longo, J. (2020). Redox-Active Phenolic Compounds Mediate the Cytotoxic and Antioxidant Effects of Carpodesmia tamariscifolia (= Cystoseira tamariscifolia). Chemistry & biodiversity, 17(7), e2000121.
6. Vizetto-Duarte, C., Custódio, L., Acosta, G., Lago, J. H., Morais, T. R., de Sousa, C. B., Varela, J. (2016). Can macroalgae provide promising anti-tumoral compounds? A closer look at Cystoseira tamariscifolia as a source for antioxidant and anti-hepatocarcinoma compounds. PeerJ, 4, e1704.
7. Gouveia, V., Seca, A. M., Barreto, M. C., & Pinto, D. C. (2013). Di-and sesquiterpenoids from Cystoseira genus: structure, intra-molecular transformations and biological activity. Mini reviews in medicinal chemistry, 13(8), 1150-1159.
8. Mesguiche, V., Valls, R., Piovetti, L., & Banaigs, B. (1997). Meroditerpenes from

Cystoseira amentacea var. Stricta collected off the Mediterranean coasts. Phytochemistry, 45(7), 1489-1494.
9. Valls, R., Piovetti, L., & Praud, A. (1993). The use of diterpenoids as chemotaxonomic markers in the genus Cystoseira. Hydrobiologia, 260(1), 549-556.
10. Amico, V., Piattelli, M., Cunsolo, F., Neri, P., & Ruberto, G. (1989). Two epimeric, irregular diterpenoid toluquinols from the brown alga Cystoseira stricta. Journal of natural products, 52(5), 962-969.
11. Bennamara, A., Abourriche, A., Berrada, M., Charrouf, M. H., Chaib, N., Boudouma, M., & Garneau, F. X. (1999). Methoxybifurcarenone: an antifungal and antibacterial meroditerpenoid from the brown alga Cystoseira tamariscifolia. Phytochemistry, 52(1), 37-40.
12. Abdoul-Latif, F. M., Oumaskour, K., Boujaber, N., Ainane, A., Mohamed, J., & Ainane, T. (2021). Formulations of a cosmetic product for hair care based on extract of the microalga Isochrysis galbana: in vivo and in vitro activities. Journal of Analytical Sciences and Applied Biotechnology, 3(1), 15-19.
13. Amico, V., Piattelli, M., Neri, P., Ruberto, G., & Mayol, L. (1986). Novel metabolites from the marine genus cystoseira-application of two-dimensional 1H-13C correlation to the structure elucidation. Tetrahedron, 42(21), 6015-6020.
14. Valls, R., Mesguiche, V., Piovetti, L., Prost, M., & Peiffer, G. (1996). Meroditerpenes from the brown alga Cystoseira amentacea var. stricta collected off the French mediterranean coast. Phytochemistry, 41(5), 1367-1371.

**Table 1.** $^{1}$H NMR spectral data of compounds A, B and C (TMS as int. standard)*.

| H | A<br>250 MHz † (CDCl3) | B<br>200 MHz ‡ (CDCl3) | C<br>300 MHz (CDCl3) |
|---|---|---|---|
| 3' | 6.58 } AB (3) | 6.45 dt (2.6, 1.8) | 6.60 dt (2.6, 1.8) |
| 5' | 6.51 | 6.54 dq (2.6, 2) | 6.69 dq (2.6, 2) |
| 1 | 3.24 dd (16, 6.3)<br>3.43 dd (16, 7.7) | 3.13 d (7) | 2.9 d (7) |
| 2 | 5.38 t (7) | 5.16 t (7) | 4.87 t (7) |
| 4 | 2.69<br>2.74 } AB (15) | 2.65<br>2.75 } AB (15) | 2.65<br>2.74 } AB (15) |
| 6 | 4.35 s | 4.30 s | 4.30 s |
| 8 | 1.57 m | 1.55 m | 1.54 m |
| 9 | 1.69 m | 1.67 m | 1.63 m |
| 10 | 1.37 m | 1.37 m | 1.37 m |
| 13 | 6.02 } AB (5.5) | 6.05 } AB (5.5) | 5.79 } AB (5.5) |
| 14 | 5.65 | 5.69 | 5.69 |
| 16 | 1.32 s | 1.43 s | 1.43 s |
| 17 | 1.28 s | 1.37 s | 1.37 s |
| 18 | 0.89 s | 1.17 s | 1.17 s |
| 19 | 1.15 s | 1.11 s | 1.11 s |
| 20 | 1.76 s | 1.63 s | - |
| Me-6' | 2.21 s | 1.56 s | 2.12 s |
| OMe-4' | 3.75 s | - | - |
| OH | 4.88 s | - | - |
| OMe-3 | - | - | 3.52 s |

*Chemical shifts are S values; coupling constants (J in parentheses) are given in Hz; assignments were confirmed by decoupling and 2D NMR experiments (COSY 1H-1H, HCCORR).
†: $^{1}$H NMR data of ref. [13] added for comparison.
‡: $^{1}$H NMR data of ref. [14] added for comparison.

**Table 2.** $^{13}C$ NMR spectral data of compounds A, B and C (TMS as int. standard).

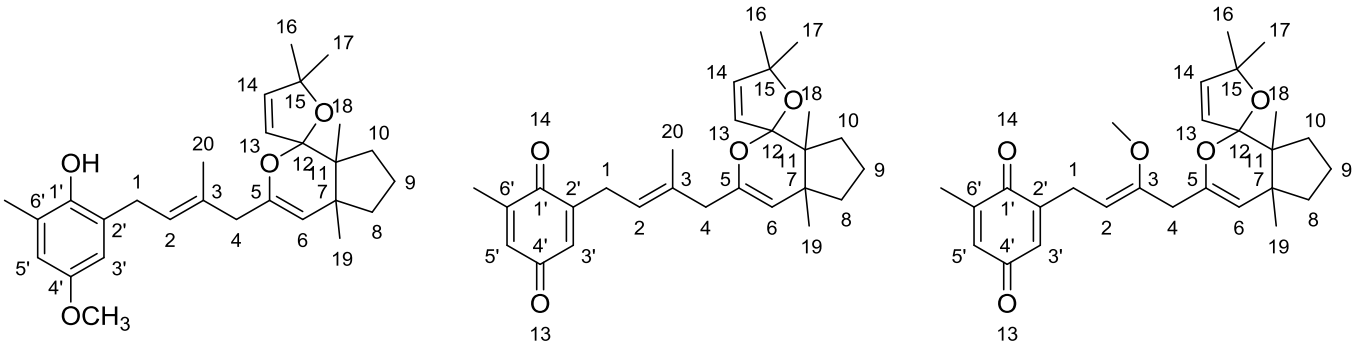
| C | A 62.5 MHz † (CDCl3) | | B 50 MHz ‡ (CDCl3) | | C 50 MHz (CDCl3) | |
|---|---|---|---|---|---|---|
| 1' | 146.5 | C | 188.0 | C=O | 188.0 | C=O |
| 2' | 136.0 | C | 148.3 | C | 148.3 | C |
| 3' | 114.0 | CH | 132.4 | CH | 132.4 | CH |
| 4' | 153.3 | C | 187.9 | C=O | 187.9 | C=O |
| 5' | 113.0 | CH | 133.3 | CH | 133.3 | CH |
| 6' | 127.0 | C | 146.2 | C | 146.2 | C |
| 1 | 30.8 | $CH_2$ | 27.7 | $CH_2$ | 27.7 | $CH_2$ |
| 2 | 123.8 | CH | 119.0 | CH | 119.0 | CH |
| 3 | 126.0 | C | 138.3 | C | 138.3 | C |
| 4 | 45.1 | $CH_2$ | 40.1 | $CH_2$ | 40.1 | $CH_2$ |
| 5 | 147.1 | C | 147.0 | C | 147.0 | C |
| 6 | 109.0 | CH | 109.5 | CH | 109.5 | CH |
| 7 | 43.2 | C | 43.2 | C | 43.2 | C |
| 8 | 40.5 | $CH_2$ | 40.4 | $CH_2$ | 40.4 | $CH_2$ |
| 9 | 20.4 | $CH_2$ | 20.4 | $CH_2$ | 20.4 | $CH_2$ |
| 10 | 36.1 | $CH_2$ | 36.2 | $CH_2$ | 36.2 | $CH_2$ |
| 11 | 46.3 | C | 46.2 | C | 46.2 | C |
| 12 | 115.0 | C | 115.1 | C | 115.1 | C |
| 13 | 140.0 | CH | 140.0 | CH | 140.0 | CH |
| 14 | 126.8 | CH | 127.0 | CH | 127.0 | CH |
| 15 | 88.0 | C | 88.0 | C | 88.0 | C |
| 16 | 26.3 | $CH_3$ | 22.8 | $CH_3$ | 22.8 | $CH_3$ |
| 17 | 28.7 | $CH_3$ | 28.8 | $CH_3$ | 28.8 | $CH_3$ |
| 18 | 20.2 | $CH_3$ | 26.3 | $CH_3$ | 26.3 | $CH_3$ |
| 19 | 22.7 | $CH_3$ | 20.3 | $CH_3$ | 20.3 | $CH_3$ |
| 20 | 16.5 | $CH_3$ | 16.2 | $CH_3$ | - | |
| Me-6' | 16.2 | $CH_3$ | 15.6 | $CH_3$ | 15.6 | $CH_3$ |
| OMe-4' | 53.6 | $CH_3$ | - | | - | |
| OMe-3 | - | | - | | 55.8 | $CH_3$ |

†: $^1H$ NMR data of ref. [13] added for comparison.
‡: $^1H$ NMR data of ref. [14] added for comparison.

**Table 3.** Antibacterial activity exhibited by MIC of compound C.

| Strain | MIC (mg/ml) |
|---|---|
| *Cutibacterium acnes* | 1.322 ± 0.085 |
| *Staphylococcus epidermidis* | 0.985 ± 0.068 |

**Figure 1.** Structures of compounds A, B and C.




A: Cystoketal B: Cystoketal quinone C: Methoxycystoketal quinone