Three New Unsaturated Fatty Acids from the Marine Green Alga *Ulva fasciata* Delile

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From the dichloromethane extract of the marine green alga *Ulva fasciata* Delile, collected from the Mediterranean coast of Egypt, three new fatty acids, namely, (*E*)-11-oxo-octadeca-12-enoic acid (**1a**), (*E*)-11-hydroxy-octadeca-12-enoic acid (**2a**) and 6-hydroxy-oct-7-enoic acid (**3a**) together with cholesterol were isolated. Analysis of the unpolar part of the extract using GC-MS detected the existence of further ten compounds, namely, dimethylsulfoxide, dimethylsulfone, phenylacetamide, 6,10,14-trimethyl-pentadecan-2-one, 8-heptadecene, dodecane, tridecane, 4-oxo-pentanoic acid, hexadecanoic acid, and the naturally new 1,1′-bicyclohexyl. Structures of the isolated compounds **1a** – **3a** were confirmed by spectroscopic analyses including mass spectra (EI-MS, HR/ESI-MS), 1D and 2D NMR experiments, and by the synthetic conversion into their corresponding methyl esters **1b** – **3b**. The algal extract and its components were comparatively examined against several pathogenic microorganisms, and brine shrimps for cytotoxicity.

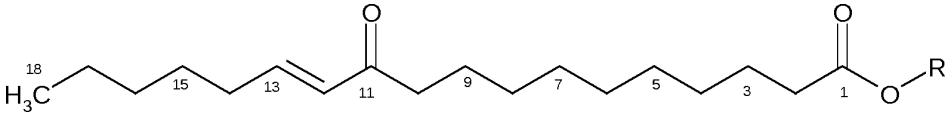
Key words: Marine Algae, *Ulva fasciata*, New Fatty Acids, GC-MS, Biological Activity, Cytotoxicity

Introduction

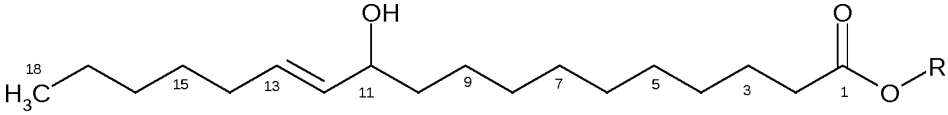
In comparison with terrestrial plants, the use of marine algae in folk medicine is very restricted. More than 150,000 macro-algae or seaweed species are found in the oceans and seas of the globe, however, only a few of them were identified [1]. The Egyptian marine habitats are known to be favored with a rich variety and abundance of marine macrophytes (Chlorophyta, Phaeophyta, Rhodophyta, and Embryophyta) [2], despite of their rather limited investigations for biological activities in such region carried out to date [3 – 8]. During the past 40 years, marine algae received a lot of attention as potential sources of compounds possessing a wide range of biological activities including antimicrobial, antiviral, anti-inflammatory, immunotropic, antitumor, as well as ichthyotoxic properties [1, 9 – 16]. These features may be primarily related to the high content of different glycolipids [17].

Polyunsaturated fatty acids (PUFAs) are unique features of lipids of marine origin and have considerable health and economic significance [18 – 20]. Some studies have recognized the vital role of conjugated fatty acids as bioactive molecules in the treatment of tumors and other cancer-related problems, with varying degree of cytotoxic effects on the cancer cells [21 – 25]. Moreover, long-chain unsaturated fatty acids, *e. g.* linoleic acid, oleic acid, and linolenic acid, show antibacterial activity and are the key ingredients of antimicrobial food additives and some antibacterial herbs [26]. They are bactericidal to important pathogenic microorganisms, including methicillin-resistant *Staphylococcus aureus*, *Helicobacter pylori*, and mycobacteria [27 – 32]. Despite of the enormous marine sources, there have been not many studies concerning the occurrence of conjugated fatty acids in the seaweeds [18].

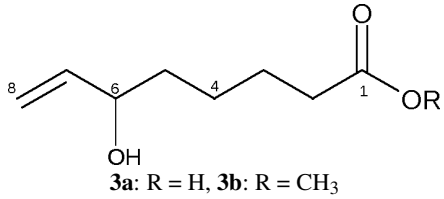
The Mediterranean coast of Egypt is rich in various green algae and dominated by *Ulva* [33], however, only few investigations have dealt with the biological activities of green algae. For *Ulva fasciata* Delile, active substances appear to be still unknown. So, the chemistry of this alga, collected from Abu-



1a: R = H; **1b**: R = CH_3



2a: R = H; **2b**: R = CH_3



3a: R = H, **3b**: R = CH_3

Qir along the Mediterranean coast, Egypt, was extensively studied in our search for bioactive marine compounds. This led to the isolation of three new fatty acids, namely (*E*)-11-oxo-octadeca-12-enoic acid (**1a**), (*E*)-11-hydroxy-octadeca-12-enoic acid (**2a**), and 6-hydroxy-oct-7-enoic acid (**3a**), along with cholesterol. The structures of the new fatty acids **1a** – **3a** were intensively studied using NMR and MS spectroscopic data, and further confirmed by the conversion of the compounds into the corresponding methyl esters **1b** – **3b**. The algal extract and its bioactive constituents were tested against a set of pathogenic microorganisms. For *in vitro* cytotoxicity, they were examined against brine shrimps and the breast carcinoma tumor cell line MCF7.

Results and Discussion

The structure of cholesterol was established by NMR and MS data and by comparison with literature data [34 – 37]. GC-MS analysis of the unpolar fractions revealed the existence of ten unpolar oily components, dimethylsulfoxide [38], dimethylsulfone [39], phenylacetamide [40], 6,10,14-trimethyl-pentadecan-2-one, phytone [41], 8-heptadecene [42], dodecane [39], tridecane, 4-oxo-pentanoic acid, levulinic acid [43], and hexadecanoic acid [44], in addition to the naturally new 1,1′-bicyclohexyl. The GC-MS data of these compounds are listed in Table 1, the physico-chemical properties of the new fatty acids (*E*)-11-oxo-octadeca-12-enoic acid (**1a**), (*E*)-11-hydroxy-octadeca-12-enoic acid (**2a**) and 6-hydroxy-oct-7-enoic acid (**3a**) are described in Table 2.

Table 1. GC-MS analysis for the combined unpolar fractions.

Name	R_t (min)	M. F.	M. Wt.
Dimethylsulfoxide	5.03	C_2H_6OS	78
Dimethylsulfone	6.29	$C_2H_6OS_2$	94
4-Oxo-pentanoic acid	8.38	$C_5H_8O_3$	116
Dodecane	10.76	$C_{12}H_{26}$	170
Tridecane	12.20	$C_{13}H_{28}$	184
1,1′-Bicyclohexyl	12.54	$C_{12}H_{22}$	166
Phenylacetamide	13.59	C_8H_9NO	135
6,10,14-Trimethyl-pentadecan-2-one	18.77	$C_{18}H_{36}O$	268
8-Heptadecene	16.98	$C_{17}H_{34}$	238
Hexadecanoic acid	19.86	$C_{16}H_{32}O_2$	256

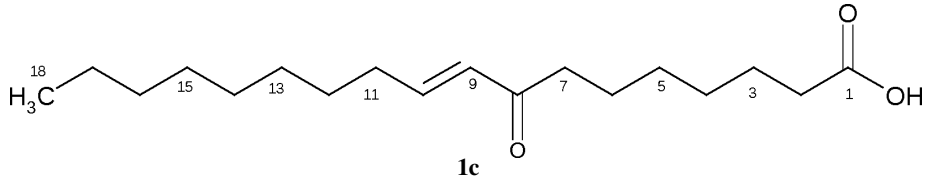
*(E)-11-Oxo-octadeca-12-enoic acid (**1a**)*

Compound **1a** was obtained as a colorless oil with a UV absorbance evident during TLC, which on spraying with anisaldehyde/sulfuric acid turned violet and later changed to blue. The molecular weight (296 Dalton) could not be determined by EI and CI MS, however, the corresponding molecular formula was established by HRMS ((+)-ESI) as $C_{18}H_{32}O_3$. Furthermore, the molecular weight of the corresponding methyl ester **1b** was deduced to be 310 Dalton according to DCI MS (see the Experimental Section), and the HRMS (ESI) resulted in the corresponding molecular formula $C_{19}H_{34}O_3$.

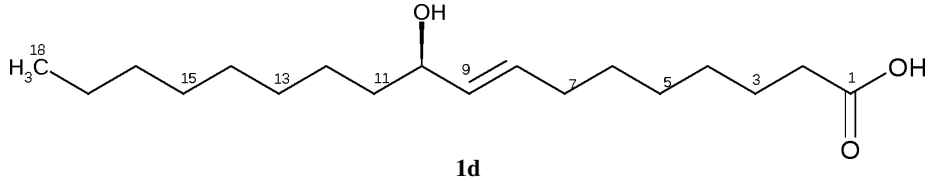
The ^{1}H NMR spectra of **1a** revealed two olefinic protons at δ = 6.83 and 6.09 with a *trans* coupling of $J \sim 15.7$ Hz. Two triplets and one quartet (each of 2H) for three methylene groups, most likely attached to sp^2 systems were visible at δ = 2.53, 2.34 and 2.21 ($J \sim$ 7.4 – 6.9 Hz). Additionally, three broad multiplets were detected in the region between δ = 1.71 – 1.55, 1.47 and 1.36 – 1.21 for 4H, 2H and 14H, corresponding probably to two, one and seven methylene groups, respectively. This revealed the existence of a long poly-

Table 2. Physico-chemical properties of the new fatty acids **1a** – **3a**.

	1a	**2a**	**3a**
Appearance	Colorless oil	Colorless oil	Colorless oil
R_f	0.60 (DCM/2 % MeOH)	0.43 (DCM/2 % MeOH)	0.21 (DCM/2 % MeOH)
Molecular formula	$C_{18}H_{32}O_3$	$C_{18}H_{34}O_3$	$C_8H_{14}O_3$
DCI-MS (NH_3): m/z (%)	–	–	158 ($[M–H_2O+NH_4]^+$, 15), 176 ($[M+NH_4]^+$, 92), 316 ($[2M–H_2O+NH_4]^+$, 36), 334 ($[2M+NH_4]^+$, 100)
EI-MS: m/z (%)	224 (6), 149 (4), 123 (96), 58 (22), 43 (100), 41 (12)	280 ($[M–H_2O]^+$, 9), 252 (6), 213 (8), 209 (16), 195 (20), 185 (26), 168 (43), 155 (14), 141 (46), 127 (68), 109 (26), 98 (46), 81 (43), 67 (47), 57 (100), 55 (90), 41 (44)	140 ($[M–H_2O]$, 18), 124 (6), 112 (56), 97 (15), 84 (38), 73 (100), 57 (98), 41 (31)
HRMS ((+)-ESI): (m/z)			
Found	297.24242 ($C_{18}H_{33}O_3$), 319.22437 ($C_{18}H_{32}O_3Na$)	321.24005 ($C_{18}H_{34}O_3Na$)	181.08351 ($C_8H_{16}O_3Na$)
Calcd.	297.24241 ($C_{18}H_{33}O_3$), 319.22435 ($C_{18}H_{32}O_3Na$)	321.24002 ($C_{18}H_{34}O_3Na$)	181.08352 ($C_8H_{16}O_3Na$)
$[\alpha]_D^{20}$	–	+2 (c = 0.1, MeOH)	−6 (c = 0.15, MeOH)
UV/Vis: λ_{max} (MeOH) nm ($\log \varepsilon$)	neutral: 226 (4.12); acidic: 225 (4.12); basic: 226 (4.12)	neutral: 202 (3.79), 276 (sh, 3.08); acidic: 201 (3.72), 276 (sh, 2.87); basic: 222 (3.35); 276 (sh, 2.87)	basic: 224 (2.63)



1c



1d

methylene chain. Finally, a methyl triplet ($J \sim 7.3$ Hz) appeared at $\delta = 0.88$.

Based on the ^{13}C NMR spectrum and HMQC experiments (Table 3), compound **1a** displayed eighteen carbon signals, among them one carbonyl ($\delta \sim 201.1$) of an enone system, one acid carbonyl ($\delta = 179.6$) and two olefinic carbons ($\delta_C = 147.5$; $\delta_H = 6.83$, and $\delta_C = 130.3$; $\delta_H = 6.09$). Moreover, three sp^2-bound methylene carbons were located at $\delta = 40.0$ ($\delta_H = 2.53$), 34.0 ($\delta_H = 2.34$) and 32.4 ($\delta_H = 2.21$). The remaining eleven carbon signals were detected in the region of $\delta = 32 \sim 22.4$, corresponding to an elongated chain of 10 methylenes and a terminal methyl group ($\delta = 14.0$). According to these NMR data and the molecular formula, the fatty acid **1a** contains an enone system (-CH_2–CH=CH–CO–CH_2-), a terminal ethyl and a free carboxylic acid group. In a search in different data bases, *i. e.* AntiBase [34], the Dictionary of Natural Products (DNP) [39] and Chemical Abstract, only 8-oxo-9-octadecenoic acid (**1c**) was revealed. However, comparison of the spectroscopic data with the literature [25] showed clear differences.

HMBC and H, H COSY experiments (Fig. 1) revealed the existence of four methylene groups between the enone system and the terminal methyl group. Respectively, the ketone carbonyl ($\delta = 201.1$) was established at 11-position, and the remaining nine methylene carbons are located between this carbonyl and the terminal acid group ($\delta = 179.6$). Thus, compound **1a** was determined as (*E*)-11-oxo-octadeca-12-enoic acid. The structure was supported by the ^1H NMR data of the respective methyl ester **1b** with a 3H singlet signal

Table 3. ^{13}C (125 MHz) and ^{1}H NMR (300 MHz, *J* in Hz) data of the three new fatty acids **1a** – **3a** in $CDCl_3$.

No.	1a		2a		3a	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	179.6	–	174.4	–	179.2	–
2	34.0	2.34 (t, 7.4)	33.6	2.18 (t, 6.7)	34.0	2.34 (m)
3	24.6	1.71 – 1.55 (m)	24.5	1.49 (m)	24.7	1.64 (m)
4	29.0	1.36 – 1.21 (m)	28.5	1.40 – 1.20 (m)	24.5	1.42 (m)
5	29.1	1.36 – 1.21 (m)	29.0	1.40 – 1.20 (m)	36.4	1.54 (m)
6	29.3	1.36 – 1.21 (m)	28.8	1.40 – 1.20 (m)	72.9	4.12 (q, 6.4)
7	32.3	1.36 – 1.21 (m)	28.8	1.40 – 1.20 (m)	140.8	5.85 (m)
8	28.7	1.36 – 1.21 (m)	28.4	1.40 – 1.20 (m)	114.8	5.21 (d, 17.2), 5.10 (d, 10.4)
9	24.3	1.71 – 1.55 (m)	24.9	1.40 – 1.20 (m)		
10	40.0	2.53 (t, 7.3)	37.4	1.40 – 1.20 (m)		
11	201.2	–	70.8	3.84 (m)		
12	130.3	6.09 (d, 15.7)	128.9	5.49 (m)		
13	147.5	6.83 (m)	134.5	5.34 (m)		
14	32.4	2.21 (q, 6.9)	31.5	1.96 (m)		
15	27.7	1.47 (m)	28.7	1.40 – 1.20 (m)		
16	31.6	1.36 – 1.21 (m)	31.2	1.40 – 1.20 (m)		
17	22.4	1.36 – 1.21 (m)	22.0	1.40 – 1.20 (m)		
18	14.0	0.88 (t, 7.3)	13.8	0.86 (t, 6.4)		

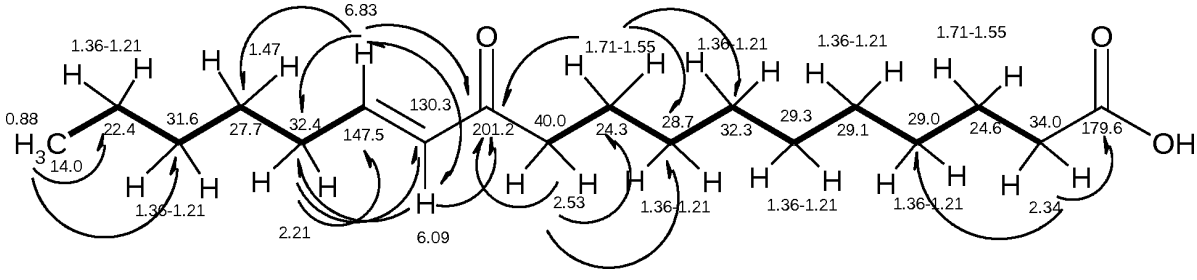


Fig. 1. HMBC → and H,H COSY (↔, ▬) connectivities of (*E*)-11-oxo-octadeca-12-enoic acid (**1a**).

at $\delta = 3.67$. Both the fatty acid **1a** and the ester **1b** are reported here for the first time. α,β-Enones are highly cytotoxic and play vital roles as aldehyde dehydrogenase inhibitors [25].

*(E)-11-Hydroxy-octadeca-12-enoic acid (**2a**)*

Compound **2a** was obtained as a colorless oil as well and turned also violet and later blue with anisaldehyde/sulfuric acid, however, it was not UV absorbing in TLC. The DCI MS did not show a *pseudo*-molecular ion, however, EI MS displayed an ion peak at *m/z* = 280 with 18 amu less than the molecular ion, due to water elimination from the parent molecule. The molecular formula of **2a** was determined by HRMS ((+)-ESI) as $C_{18}H_{34}O_3$ and further confirmed by the HRESI MS data and the methoxy singlet at $\delta_H = 3.67$ of the methyl ester **2b** in the ^{1}H NMR spectrum.

Based on the molecular formula, compound **2a** is closely related to **1a**, with a molecular weight higher by 2 amu. In the ^{13}C NMR spectrum (Table 3) the ketone carbonyl resonance ($\delta = 201.2$) of **1a** had disappeared, while an oxygenated methine ($\delta = 70.8$) was detected. This corresponds to the reduction of the ketone carbonyl of **2a**. Correspondingly, the olefinic protons of **2a** appeared in the ^{1}H NMR spectra as multiplets at $\delta = 5.49$ and 5.34, and an additional oxymethine signal was visible at $\delta = 3.84$. With respect to the literature [45], the acid **2a** is related, but not identical with 9-hydroxy-10-octadecenoic acid (**1d**).

A full signal assignment was achieved by H,H COSY and HMBC experiments (Fig. 2). Accordingly, the oxymethine carbon is directly attached to the olefinic double bond. As in **1a**, only five terminal carbons including a methyl group ($\delta_C = 13.8$, $\delta_H = 0.86$) were recognized. Respectively, the hydroxymethine carbon ($\delta = 70.8$) was fixed at the 11-position, and the double bond ($\delta = 128.9$ and 134.5) located at the neighboring carbons 12, 13. The carboxyl group ($\delta = 174.4$) is in a terminal position and connected with

Table 4. Antimicrobial activities of compounds **1a** – **3a** compared with the algal extract using the agar diffusion method.

	Agar diffusion test (40 µg/disc (∅ 9 mm); diameter of inhibition zones in [mm])										
Compounds	BS[a]	SA[b]	SV[c]	EC[d]	CA[e]	MM[f]	CV[g]	CS[h]	SS[i]	RS[j]	PU[k]
Ulva fasciata extract	0	13	20	10	0	0	0	0	0	0	0
1a	0	11	0	0	0	0	13	13	12	0	0
2a	0	14	0	11	0	0	0	0	0	0	0
3a	0	0	0	0	0	0	0	0	0	0	0

[a] *Bacillus subtilis*, [b] *Staphylococcus aureus*, [c] *Streptomyces viridochromogenes* (Tü 57), [d] *Escherichia coli*, [e] *Candida albicans*, [f] *Mucor miehi*, [g] *Chlorella vulgaris*, [h] *Chlorella sorokiniana*, [i] *Scenedesmus subspicatus*, [j] *Rhizoctonia solani*, [k] *Pythium ultimum*.

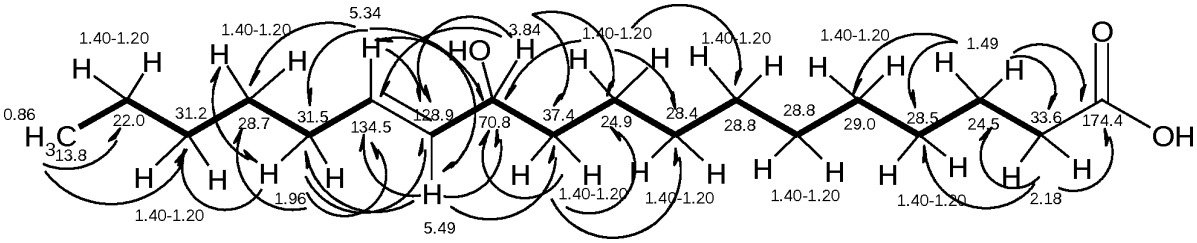


Fig. 2. HMBC (→) and H, H COSY (↔, ▬) connectivity of (*E*)-11-hydroxy-octadeca-12-enoic acid (**2a**).

a chain of 10 methylene groups. This results in (*E*)-11-hydroxy-octadeca-12-enoic acid (**2a**), which is a new unsaturated hydroxyfatty acid.

*6-Hydroxy-oct-7-enoic acid (**3a**)*

Compound **3a** was obtained as another colorless oil with similar chromatographic properties as **2a**. Its molecular weight was deduced by CI MS as 158 Dalton, while on EI MS, an ion peak appeared at *m/z* = 140 as a result of the expulsion of water from the parent molecule. This pointed to a hydroxyl-propene system as in **2a**. The molecular formula of **3a** ($C_8H_{14}O_3$) was established by HRMS ((+)-ESI). The formation of a methyl ester (MW 172 by CI MS) confirmed an acid with the molecular weight *m/z* = 158 and the formula $C_8H_{14}O_3$.

The ^{1}H NMR spectrum of **3a** indicated the existence of a terminal olefinic double bond, the respective three protons appearing at δ = 5.85, 5.21 and 5.10. According to the HMQC experiment, the latter two protons belonged to a methylene carbon (δ = 114.8). Additionally, an oxygenated methine proton (δ = 4.12) and four 2H multiplets (δ = 2.34∼1.41) of methylene groups were detected. The ^{13}C NMR and HMQC spectra (Table 3) indicated eight carbon signals, the carbonyl of an acid (δ 179.2), and two olefinic signals of the double bond at δ_{CH} = 140.8 and δ_{CH_2} = 114.8. In the aliphatic region, signals of an oxygenated carbon (δ = 72.9) and of four methylenes (δ = 34.0∼24.5) were observed.

These data, the molecular formula and further 2D NMR experiments (Fig. 3) established this compound as 6-hydroxy-oct-7-enoic acid (**3a**), which is also a new fatty acid.

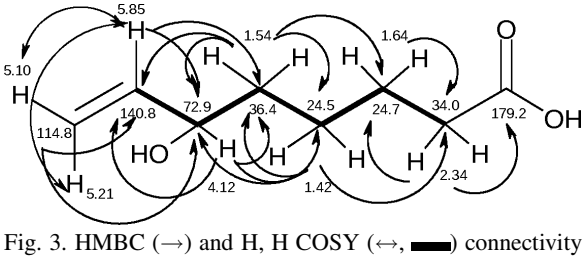


Fig. 3. HMBC (→) and H, H COSY (↔, ▬) connectivity of 6-hydroxy-oct-7-enoic acid (**3a**).

Biological activities

The activity patterns displayed by the crude extract of *U. fasciata* as well as the isolated compounds **1a** – **3a** against the test organisms were not consistent (Table 4). Whereas the crude extract was largely active against *Streptomyces viridochromogenes*, *S. aureus* and *E. coli*, the fatty acid **1a** showed moderate activities against the Gram positive *Staphylococcus aureus* and the microalgae *Chlorella vulgaris*, *Chlorella sorokiniana*, and *Scenedesmus subspicatus*. Fatty acid **2a** showed moderate and weak activities against *S. aureus* and *E. coli*, respectively, but compound **3a** was inactive against all tested organisms. The crude extract and the isolated new fatty acids **1a** – **3a** showed weak cytotoxic activity against brine shrimps (Table 5).

The *Ulva fasciata* extract was additionally examined for *in vitro* cytotoxicity against the breast carci-

Table 5. Cytotoxic activities of **1a** – **3a** in comparison with the algal extract against brine shrimps.

Component	Brine shrimp lethality at 10 μg mL^{-1}
Ulva fasciata extract	2.1 %
1a	1.6 %
2a	1.5 %
3a	6.4 %

Table 6. Antitumor activity of *U. fasciata* extract against breast carcinoma (MCF7) compared with doxorubicin.

Component	Cell line MCF7 (IC_{50}, μg)
Ulva fasciata extract	1.07
Doxorubicin	0.7

noma tumor cell line MCF7 in comparison with doxorubicin as reference (Table 6). The extract was found to reveal a potent antitumor activity at IC_{50} of 1.07 μg, which is in the range of the standard (IC_{50} = 0.7 μg).

Experimental Section

Optical rotations: Polarimeter Perkin-Elmer, model 343. UV/Vis spectra were recorded on a Perkin-Elmer Lambda 15 UV/Vis spectrometer. NMR spectra were measured on a Bruker AMX 300 (300.135 MHz), a Varian Unity 300 (300.145 MHz) and a Varian Inova 600 (150.820 MHz) spectrometer. DCI-MS: Finnigan MAT 95 A, 200 eV, reactant gas NH_3. Electrospray ionization mass spectrometry (ESI-MS): Finnigan LCQ ion trap mass spectrometer. High-resolution mass spectra (HRMS) were recorded by ESI MS on an Apex IV 7 Tesla Fourier-Transform Ion Cyclotron Resonance Mass Spectrometer (Bruker Daltonics, Billerica, MA, USA). EI mass spectra were recorded on a Finnigan MAT 95 spectrometer (70 eV) with perfluorkerosine as reference substance for EI HRMS. For GC-MS, a Trace GC-MS Thermo Finnigan instrument was used, equipped with a capillary column CP-Sil 8 CB for amines (length: 30 m; inside diameter: 0.25 mm; outside diameter: 0.35 mm; film thickness: 0.25 μm); ionization mode EI (70 eV). The analysis was carried out at a programmed temperature: initial temperature 40 °C (kept for 1 min), then increasing at a rate of 10 °C/min to 280 °C (kept for 10 min); the injector temperature was set at 250 °C and the detector temperature at 250 °C; He as a carrier gas at a flow rate of 1 mL min^{-1}, total run time 27 min, injection volume 0.2 μL. Flash chromatography was carried out on silica gel (230 – 400 mesh). Size-exclusion chromatography was done on Sephadex LH-20 (Pharmacia). R_f values were measured on Polygram SIL F/UV_{254} (Merck, pre-coated sheets).

Collection and identification of algal material

Samples of *Ulva fasciata* Delile (green alga) were collected during spring 2007 from Abu-Qir about 42 km east of Alexandria, where this alga predominates on the Mediterranean coast of Egypt. The alga was identified according to Nasr (1940) [46] and Abou-ElWafa (2005) [33].

Preparation of samples

The collected samples were cleaned up from epiphytes and the non-living matrix in running water, and rinsed many times in distilled water. The samples were then spread on string nets and allowed to dry in air. The air-dried samples were ground and stored in suitable closed bottles at room temperature.

Extraction and isolation of the active constituents

Two kg of air-dried algal material was extracted in a Soxhlet apparatus for ~10 h using dichloromethane. The dichloromethane extract was filtered and concentrated *in vacuo* at 40 °C to dryness. The dark green crude extract (8.3 g) was fractionated on Sephadex LH-20 (DCM/40 % MeOH) delivering five fractions: I (3.2 g), II (1.8 g), III (1.2 g), IV (0.8 g) and V (1.3 g). Fraction I displayed no interesting components. Fraction II was dissolved in methanol and extracted by cyclohexane. The cyclohexane extract (0.8 g) was submitted to GC-MS analysis, detecting the existence of dimethylsulfoxide, dimethylsulfone, phenylacetamide, and 6,10,14-trimethyl-pentadecan-2-one. Fractions III, IV and V were applied individually to Sephadex LH-20 (DCM/40 % MeOH). The eluates from the three fractions were combined according to TLC monitoring into two sub-fractions A (1.1 g) and B (1.6 g). Sub-fraction A was applied to a silica gel column (cyclohexane-DCM), followed by purification on a Sephadex LH-20 column (DCM/40 % MeOH) to afford cholesterol as colorless solid (17.1 mg). Further unpolar components UL2, UL3, UL4, UL7 and UL8 from sub-fraction A were identified by GC-MS analysis as 8-heptadecene, dodecene, tridecane, 1,1′-bicyclohexyl, 4-oxo-pentanoic acid and hexadecanoic acid (Table 1). Similarly, sub-fraction B was applied to a silica gel column (cyclohexane-DCM-MeOH) followed by a Sephadex LH-20 column (DCM/40 % MeOH) to deliver three colorless oils of (*E*)-11-oxo-octadeca-12-enoic acid (**1a**, 11.0 mg), (*E*)-11-hydroxy-octadeca-12-enoic acid (**2a**, 10.2 mg) and 6-hydroxy-oct-7-enoic acid (**3a**, 8.1 mg). Further three unpolar fractions UL5, UL10 and UL12 were combined and analyzed by GC-MS (Table 1).

*11-Oxo-octadeca-12-enoic acid methyl ester (**1b**)*

3 mg of 11-oxo-octadeca-12-enoic acid (**1a**, dissolved in 3 mL dry dichloromethane) was treated with 0.7 mL of an ethereal diazomethane solution at 0 °C. The reaction mixture was evaporated *in vacuo*, yielding **1b** as a colorless oil, (3.1 mg). It is UV absorbing on TLC and turns green on

spraying with anisaldehyde/sulfuric acid and heating. – R_f = 0.96 (CH_2Cl_2/2 % MeOH). – ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 6.82 (m, 1H), 6.08 (d, 1H, J = 15.9 Hz), 3.67 (s, 3H, O-CH_3), 2.32 (t, 2H, J = 7.3 Hz), 2.30 (t, 2H, J = 7.3 Hz), 2.10 (q, 2H, J = 7.0 Hz), 1.60 (m, 4H), 1.47 (m, 2H), 1.29 (brs, 14H), 0.88 (t, 3H, J = 7.5 Hz). – DCIMS (NH_4) *m/z* (%) = 328, $[M+NH_4]^+$, (100), 638, $[2M+NH_4]^+$, (7). – HRMS ((+)-ESI): *m/z* = 311.25820 (calcd. 311.25807 for $C_{19}H_{35}O_3$, $[M+H]^+$). – HRMS ((+)-ESI): *m/z* = 333.24020 (calcd. 333.24002 for $C_{19}H_{34}O_3Na$, $[M+Na]^+$).

*(E)-11-Hydroxy-octadeca-12-enoic acid methyl ester (**2b**)*

As for **1b**, 2 mg of (*E*)-11-hydroxy-octadeca-12-enoic acid (**2a**) was methylated, affording 2.05 mg of **2b** as a colorless oil. On TLC, it is not UV absorbing and turns pink and later blue on spraying with anisaldehyde/sulfuric acid. – R_f = 0.88 (CH_2Cl_2/2 % MeOH). – ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 5.61 (m, 1H), 5.44 (dd, 1H, J = 15.5, 7.0 Hz), 4.03 (q, 1H, J = 7.1 Hz), 3.67 (s, 3H, O-CH_3), 2.30 (t, 2H, J = 7.4 Hz), 2.02 (q, 2H, 7.2 Hz), 1.61 (m, 4H), 1.28 (brs, 18H), 0.88 (t, 3H, J = 7.4 Hz). – HRMS ((+)-ESI): *m/z* = 335.25562 (calcd. 335.25567 for $C_{19}H_{36}O_3Na$, $[M+Na]^+$).

*6-Hydroxy-oct-7-enoic acid methyl ester (**3b**)*

As for **1b**, 2.1 mg of 6-hydroxy-oct-7-enoic acid (**3a**) was treated with diazomethane at 0 °C, affording a colorless oil (2.21 mg) of **3b** with similar chromatographic properties as for **2b**. – R_f = 0.42 (CH_2Cl_2/2 % MeOH). – ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 5.86 (m, 1H), 5.23 (td, 1H, J = 17.0, 1.3 Hz), 5.11 (td, 1H, J = 10.3, 1.3 Hz), 4.14 (q, 1H, J = 6.4 Hz), 3.67 (s, 3H, O-CH_3), 2.33 (t, 2H, J = 7.4 Hz), 1.69 – 1.26 (m, 6H). – DCI MS (NH_4): *m/z* (%) = 190, $[M+NH_4]^+$, (100), 362, $[2M+NH_4]^+$, (22). – HRMS ((+)-ESI): *m/z* = 195.0992 (calcd. 195.09917 for $C_9H_{16}O_3Na$, $[M+Na]^+$).

Biological activity

Antimicrobial activity

Antimicrobial assays were conducted utilizing the disc-agar method [47] against a set of microorganisms listed in Table 4. The *Ulva fasciata* extract and the new fatty acids **1a** – **3a** were dissolved in CH_2Cl_2/10 % MeOH at a concentration of 1 mg mL^{-1}. Aliquots of 40 μL were soaked on filter paper discs (9 mm ∅, no. 2668, Schleicher & Schüll, Germany) and dried for 1 h at r. t. under sterile conditions. The paper discs were placed on agar plates inseeded with the test organisms and incubated for 24 h at 38 °C for bacterial and 48 h (30 °C) for fungal isolates, while the algal test strains were incubated at room temperature in daylight.

Brine shrimp microwell cytotoxic assay

The cytotoxic assay was performed according to Takahashi *et al*. [48]. A number of 30∼40 shrimp larvae were transferred to a deep-well microtiter plate (wells diameter 1.8 cm, depth 2 cm) filled with 0.2 mL of salt water, and the dead larvae were counted (number N). A solution of 10 μg/mL of the examined compound was added to the plate. This plate was kept at r. t. for 24 h, then the dead larvae were counted in each well under the microscope (number A). The still living larvae were killed by addition of *ca*. 0.5 mL methanol so that subsequently the total number of the animals could be determined (number G). The mortality rate M was calculated in %. Each test row was accompanied by a blind sample with pure DMSO (number B) and a control sample with 1 μg/test actinomycin D. The mortality rate M was calculated using the following formula:

$$M = \left[\frac{A - B - N}{G - N}\right] \cdot 100$$

In vitro cytotoxic activity using the SRB assay

In vitro cytotoxicity of the desired components was tested according to Skehan *et al*. [49]. Cells were placed in a 96-multiwell plate (10^4 cells per well) for 24 h before treatment with the components to be tested to allow attachment of the cells to the wall of the plate. Different concentrations of the components under test (0, 1, 2.5, 5, 10 μg mL^{-1}) were set up and added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with tested components for 48 h at 37 °C in an atmosphere with 5 % CO_2. After incubation, cells were fixed, washed and stained with Sulfo-Rhodamine-B pigment. Excess of the dye was washed off with acetic acid, while the adsorbed dye was dissolved with Tris-EDTA buffer. The color intensity was measured in an ELISA reader. The relation between surviving fraction and drug concentration was plotted to get the survival curve of each tumor cell line after application of the specified compound.

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[1] J. V. M. Lima-Filho, A. F. F. U. Carvalho, S. M. Freitas, V. M. M. Melo, *Brazilian J. Microbiology* **2002**, *33*, 311 – 313.

[2] G. R. South, A. Whittick, *Introduction to Phycology*. Blackwell Scientific Publications, Oxford, **1987**.

[3] A. F. Khaleafa, A. M. Kharboush, A. Metwalli, A. F. Mohsen, A. Serwi, *Bot. Mar.* **1975**, *18*, 163 – 165.

[4] M. E. E. El-Naggar, *Mans. Sci. Bull*. **1987**, *14*, 189 – 201.

[5] M. E. E. El-Naggar, *J. Environ. Sci*. **1996**, *11*, 105 – 118.

[6] S. S. Mabrouk, N. M. A. El-Shayeb, A. A. Hamdy, *Planta Medica* **1991**, *57*, 8 – 10.

[7] H. M. Soliman, M. E. E. El-Naggar, F. A. Badria, G. S. Abou-ElWafa, *J. Union Arab. Biol*. **2005**, *178*, 12 – 28.

[8] M. E. E. El-Naggar, H. M. Soliman, F. A. Badria, G. S. Abou-ElWafa, *N. Egypt. J. Microbiol*. **2007**, *16*, 230 – 243.

[9] P. R. Burkholder, G. M. Sharma, *Lloydia* **1969**, 32, 466 – 483.

[10] E. Ballesteros, D. Martin, M. J. Uriz, *Bot. Mar*. **1992**, *35*, 481 – 485.

[11] S. W. A. Naqvi, K. S. Y. Solimabi, L. Fernandes, C. V. G. Reddy, D. S. Bhakuni, B. N. Dhawan, *Bot. Mar*. **1980**, *24*, 51 – 55.

[12] K. L. Rinehart, Jr., P. D. Shaw, L. S. Shield, J. B. Gloer, G. C. Harbour, M. E. S. Koker, D. Samain, R. E. Schwartz, A. A. Tymiak, D. L. Weller, G. T. Carter, M. H. G. Munro, H. E. Renis, E. G. Swynenberg, D. A. Stringfellow, J. J. Vavra, J. H. Coats, G. E. Zurenko, S. L. Kuentzel, G. J. Bakus, R. C. Brusca, L. L. Craft, D. N. Young, J. L. Connor, *Pure Appl. Chem*. **1981**, *53*, 793 – 817.

[13] J. L. Reichelt, M. A. Borowitzka, *Proc. Int. Seaweed Symp*. **1984**, *11*, 158 – 168.

[14] L. M. Hodgson, *Bot. Mar*. **1984**, *27*, 387 – 390.

[15] V. J. Paul, W. Fenical, in *Bioorganic Marine Chemistry* (Ed.: P. J. Scheuer), Springer-Verlag, Berlin **1991**, pp. 1 – 29.

[16] M. H. G. Munro, R. T. Luibrand, J. W. Blunt, in *Bioorganic Marine Chemistry* (Ed.: P. J. Scheuer), Springer-Verlag, Berlin, **1991**, pp. 93 – 176.

[17] N. M. Sanina, S. N. Goncharova, E. Y. Kostetsky, *Phytochemistry* **2004**, *65*, 721 – 730.

[18] N. Bhaskar, T. Kinami, K. Miyashita, S. Park, Y. Endo, K. Fujimoto, *Z. Naturforsch*. **2004**, *59b*, 310 – 314.

[19] W. E. M. Lands, in *Nutritional Evaluation of Long Chain Fatty acid in Fish Oil* (Eds.: S. M. Barlow, M. E. Stansby) Academic Press, London, **1982**, pp. 25 – 88.

[20] A. Lopez, W. H. Gerwick, *J. Agardh. Lipids* **1987**, *22*, 190 – 194.

[21] J. Fritsche, H. Steinhart, *Fette Lipide/Lipids* **1988**, *100*, 190 – 210.

[22] R. Noguchi, Y. Ysuli, R. Suzucki, M. Hosakawa, K. Fukunaga, K. Miyashita, *Arch. Biochem. Biophys*. **2001**, *68*, 69 – 76.

[23] H. Kohno, R. Suzucki, R. Noguchi, M. Hosakawa, K. Miyashita, T. Tanaka, *Jpn. J. Cancer Res*. **2002**, *93*, 133 – 142.

[24] R. Suzucki, R. Noguchi, T. Ota, M. Abe, K. Miyashita, T. Kawada, *Lipids* **2001**, *36*, 477 – 482.

[25] H. Kawagishi, T. Miyazawa, H. Kume, Y. Arimoto, T. Inakuma, *J. Nat. Prod.* **2002**, *65*, 1712 – 1714.

[26] C. J. Zheng, J. Yoo, T. Lee, H. Cho, Y. Kim, W. Kim, *FEBS Letters* **2005**, *579*, 5157 – 5162.

[27] H. R. Knapp, M. A. Melly, *J. Infect. Dis*. **1986**, *154*, 84 – 94.

[28] M. Farrington, N. Brenwald, D. Haines, E. Walpole, *J. Med. Microbiol*. **1992**, *36*, 56 – 60.

[29] J. J. Kabara, D. M. Swieczkowski, A. J. Conley, J. P. Truant, *Antimicrob. Agents Chemother*. **1972**, *2*, 23 – 28.

[30] C. Q. Sun, C. J. O. Connor, A. M. Roberton, *FEMS Immunol. Med. Microbiol*. **2003**, *36*, 9 – 17.

[31] S. L. Hazell, D. Y. Graham, *J. Clin. Microbiol*. **1990**, *28*, 1060 – 1061.

[32] V. Seidel, P. W. Taylor, *Int. J. Antimicrob. Agents* **2004**, *23*, 613 – 619.

[33] G. S. E. Abou-ElWafa, *M. Sc. Thesis*, Faculty of Science, Mansoura University, Mansoura (Egypt) **2005**.

[34] H. Laatsch, *AntiBase* **2007**, *A Data Base for Rapid Dereplication and Structure Determination of Microbial Natural Products*, Wiley-VCH, Weinheim, **2009**; see: http://www.user.gwdg.de/~ucoc/laatsch/AntiBase.htm.

[35] V. U. Ahmad, A. H. Memon, M. S. Ali, S. Perveen, M. Shameel, *Phytochemistry* **1996**, *42*, 1141 – 1143.

[36] J. K. Volkman, C. L. Farmer, S. M. Barrett, E. L. Sikes, *J. Phycol*. **1997**, *33*, 1016 – 1023.

[37] T. Itoh, D. Sica, C. Djerassi, *J. Chem. Soc., Perkin Trans*. **1983**, *1*, 147 – 153.

[38] T. W. Pearson, H. J. Dawson, H. B. Lackey, *J. Agric. Food Chem*. **1981**, *29*, 1089 – 1091.

[39] Dictionary of Natural Products on CD-ROM, Chapman & Hall Chemical Database, **2004**.

[40] C. R. Strauss, R. W. Trainor, *Org. Prep. Proced. Int*. **1995**, *27*, 552 – 555.

[41] A. K. Baglai, L. L. Gurarii, G. G. Kuleshov, *J. Chem. Eng. Data* **1988**, *33* , 512 – 518.

[42] B. Corbier, P. Teisseire, *Recherches* **1974**, *19*, 289 – 290.

[43] M. Negwer, *Organic-Chemical Drugs and their Synonyms* (6^{th} ed.), Akademie-Verlag, Berlin, **1987**, 308.

[44] W. D. Ollis, B. T. Redman, R. J. Roberts, I. O. Sutherland, O. R. Gottlieb, M. T. Magalhaes, *Phytochemistry* **1978**, *17*, 1383 – 1388.

[45] H. Koshino, S. Togiya, T. Yoshihara, S. Sakamura, T. Shimanuki, T. Sato, A. Tajimi, *Tetrahedron Lett*. **1987**, *28*, 73 – 76.

[46] A. H. Nasr, *The marine algae of Alexandria. 1- A report on some marine algae collected from the vicinity of Alexandria*, Notes and memoirs No. 36, Government Press, Bulaq, Cairo, **1940**, pp. 33 pl. VI.

[47] P. R. Burkholder, L. M. Burkholder, L. R. Almodovar, *Bot. Mar*. **1960**, *2*, 149 – 156.

[48] A. Takahashi, S. Kurasawa, D. Ikeda, Y. Okami, T. Takeuchi, *J. Antibiot.* **1989**, *32*, 1556 – 1561.

[49] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J. T. Warren, H. Bokesch, S. Kenney, M. R. Boyd, *J. Nat. Cancer Inst*. **1990**, *82*, 1107 – 1112.