Dendrophen, a Novel Glycyrrhetyl Amino Acid from *Dendronephthya hemprichi*

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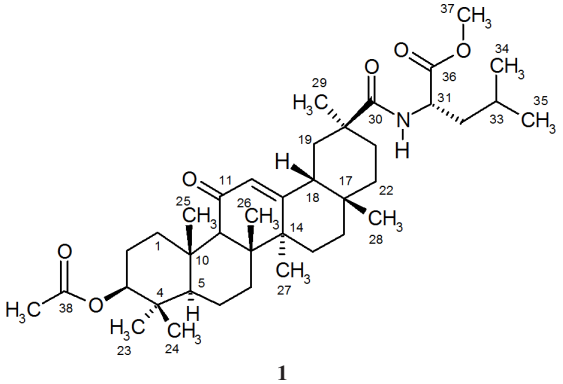
Chemical investigation of the extract of *Dendronephthya hemprichi*, collected from the Red Sea, Egypt, delivered dendrophen (**1**), the first 3β-acetoxy-glycyrrhetyl amino acid conjugate obtained from nature. Additionally, a new polyhydroxy sterol, dendrotriol (**2**), together with cholesterol and hexitol were isolated. Chromatographic separation of the low-polarity components of the *D. hemprichi* extract afforded 4-oxo-pentanoic acid, 2-methyl-acrylic acid 2-diethylaminoethyl ester (**3**), juniper camphor (**4**), and 2-octadecanone. The structures of **1** and **2** were confirmed by 1D and 2D NMR studies and mass spectrometry.

Key words: Dendrophen, Dendrotriol, *Dendronephthya hemprichi*, Biological Activity

Introduction

Soft corals are an unusually productive source of chemically interesting and biologically significant secondary metabolites [1, 2]. The vast majority of identified metabolites from soft corals are terpenoids, among which the cembranoid diterpenes are dominating [1]. Chemical investigation of soft corals of the genus *Dendronephthya* collected from various locations led to the isolation of a variety of compounds, most of them with steroid skeletons [3–5]. Eight brominated oxylipins and oxylipin glycosides were recently isolated from the Red Sea *Dendrophyllia* sp., *Dendronephthya* sp. (red and yellow variety), and *Tubipora musica* [6]. The compounds gave positive results in a brine shrimp toxicity assay. Isogosterones A–D, a group of antifouling 13,17-*seco*-steroids, were subsequently isolated from *Dendronephthya* and other species [7, 8]. Despite the fact that the chemistry of coelenterates continues to be dominated by diterpenoids and polyhydroxylated sterols, there are rare instances of the isolation of triterpenes from marine invertebrates [9]. Triterpenes containing amino acid residues are highly active as anti-HIV agents [10], but have never been reported from nature.



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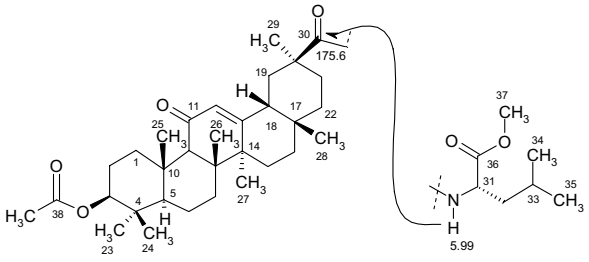
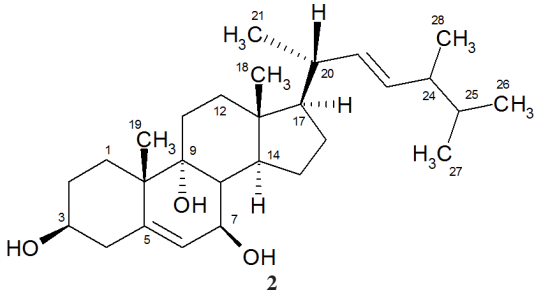
During our search for bioactive leads, we have isolated acetoxy-glycyrrhetyl-L-leucine methyl ester, dendrophen (**1**), from *Denderonephthya hemprichi*, the first triterpene amino acid obtained from nature, together with the new polyhydroxy sterol, dendrotriol (**2**). Additionally, cholesterol and hexitol were isolated, while 4-oxo-pentanoic acid, 2-methyl-acrylic acid 2-diethylaminoethyl ester (**3**), juniper camphor (**4**), and 2-octadecanone were tentatively assigned by GC-MS in the non-polar fractions of the coral extract. The two varieties of the soft coral *Denderonephthya hemprichi* (reddish orange and reddish violet) were collected



A **B**

Fig. 1. The partial structures (**A**, **B**) of dendrophen (**1**) and their connection *via* HMBC correlation.



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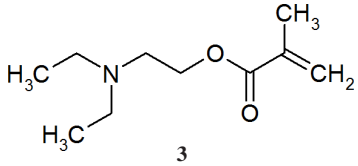

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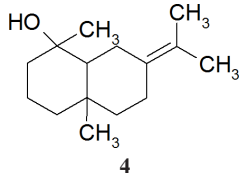

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from the Red Sea (Safaga) on the Egyptian coast. Their extracts were examined comparatively against different pathogenic microorganisms, establishing identical properties. The structures of the newly isolated compounds, dendrophen (**1**) and dendrotriol (**2**), were determined through extensive use of NMR (1D and 2D) and mass spectrometry, as well as by comparison of their data with those of related structures.

Results and Discussion

Two varieties of the soft coral *Dendronephthya hemprichi* (reddish orange and reddish violet) were individually collected from the Red Sea (Safaga) on the Egyptian coast, and subjected to morphological, biological and chemical characterizations, confirming that they were chemotaxonomically identical.

Table 1. GC-MS analysis for the unpolar fractions and components.

Name	R_t (min)	M_F	M_{Wt}
4-Oxo-pentanoic acid	8.39	$C_5H_8O_3$	116
2-Methyl-acrylic acid 2-diethylaminoethyl ester (**3**)	11.09	$C_{10}H_{19}NO_2$	185
Juniper camphor (**4**)	17.03	$C_{15}H_{26}O$	222
2-Octadecanone	20.40	$C_{18}H_{36}O$	268

Both organisms were combined and exhaustively extracted using dichloromethane-methanol. The remaining material was then dried and re-extracted with ethanol. The identical extracts were combined and fractionated by column chromatography under TLC control, eluting with *n*-hexane-dichloromethane-methanol, to afford four fractions. Purification of the middle polar fractions III and IV delivered dendrophen (**1**), dendrotriol (**2**), cholesterol and hexitol. Structures of the known metabolites, cholesterol [11] and hexitol [12–16], were established by NMR and mass spectrometry data, and confirmed by comparison with the corresponding data in the literature.

The non-polar fractions were analyzed by GC-MS with the results pointing tentatively to the existence of 4-oxo-pentanoic acid, 2-methyl-acrylic acid 2-diethylaminoethyl ester (**3**), juniper camphor (**4**) and 2-octadecanone (Table 1). The physico-chemical properties of the new compounds dendrophen (**1**) and dendrotriol (**2**) are listed in Table 2.

Dendrophen

Dendrophen (**1**) was obtained from fraction III after a sequence of chromatographic purification steps. The molecular weight of **1** was established by (+)- and (–)-ESI MS as 639 Da, and HRESI MS led to the corresponding molecular formula $C_{39}H_{61}NO_6$.

The ^{1}H NMR/HMQC spectra of **1** (Table 3) revealed the presence of an NH doublet (5.99), an olefinic methine singlet (5.74, δ_C = 128.4), a multiplet of an α-NH-CO-attached methine (4.64, δ_C = 50.4), and of an oxymethine (dd, 4.49, δ_C = 80.6) and a signal of a methoxy group (3.71, δ_C = 52.3). Ten methyl signals were revealed, among them one acetate, seven additional singlets and two doublets in the range of δ = 0.70 – 1.40. Complex signal patterns for 28 protons were found in the aliphatic range. Based on the ^{13}C NMR/HMQC spectra, ten quaternary carbons were established, among them four carbonyls belong-

Table 2. Physico-chemical properties of dendrophen (**1**) and dendrotriol (**2**).

	Dendrophen (**1**)	Dendrotriol (**2**)
Appearance	colorless solid	colorless solid
R_f	0.29[a,b]	0.38[a,c]
Staining with anisaldehyde/sulfuric acid	reddish brown	blue, turned later to brown
Molecular formula	$C_{39}H_{61}NO_6$	$C_{28}H_{46}O_3$
(+)-ESI-MS: m/z (%)	662 (15) $[M+Na]^+$, 1301 (100) $[2M+Na]^+$	
(–)-ESI-MS: m/z	638 $[M-H]^-$	
EI-MS: m/z (%)	–	412 (19) $[M-H_2O]^+$, 394 (12) $[M-2H_2O]^+$, 379 (8) $[M-(2H_2O+Me)]^+$, 365 (5), 269 (7), 251 (12), 215 (5), 159 (7), 107 (8), 95 (13), 81 (16), 69 (36), 44 (100)
HRMS ((+)-ESI): m/z		
Calcd.	640.45714 ($C_{39}H_{62}NO_6$)	453.33390 ($C_{28}H_{46}O_3Na$)
Found	640.45700, $[M+H]^+$	453.33389, $[M+Na]^+$
$[\alpha]_D^{20}$ (MeOH), deg	+165 (c = 0.18)	−17 (c = 0.06)

[a] Silica gel 60 F254, Merck; [b] *n*-hexane-CH_2Cl_2 2 : 1; [c] CH_2Cl_2-MeOH 90 : 10.

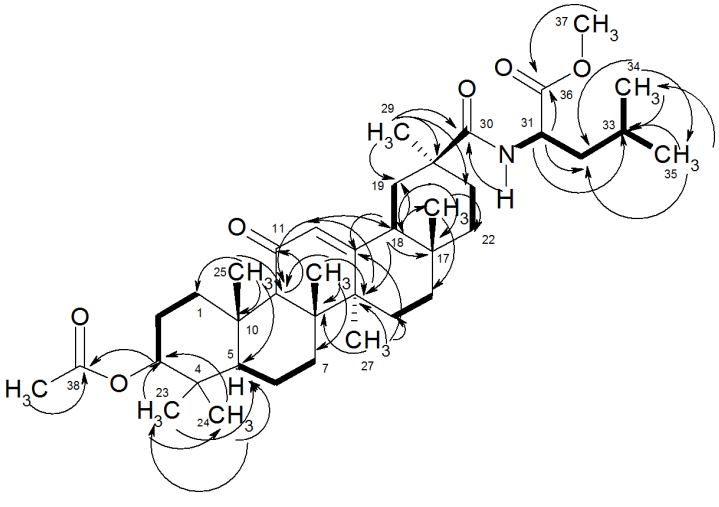

Fig. 2. H,H COSY (▬) and selected HMBC (→) connectivities of dendrophen (**1**).

ing to two esters (δ = 173.5, 171.0), an amide (δ = 175.6), a conjugated carbonyl (δ = 200.0) and a further carbonyl at δ = 169.2. The remaining six quaternary carbons were located in the sp^3 region (δ = 45.3 – 31.8). In accordance with this data, it was deduced that compound **1** is comprised of 11 methyls, 10 methylenes, 8 methines, and 10 quaternary carbons, and has ten double bond equivalents.

Based on the H,H COSY and HMBC connectivities (Figs. 1, 2), an isobutyl partial structure of leucine methyl ester (**B**) was established, where the methyl ester group (δ_H = 3.71) showed a ^3J coupling to the carbonyl at δ_C = 171.0. Moreover, the two isopropyl methyl doublets CH_3-34 (δ_C = 21.7) and CH_3-35 (δ_C = 22.9) were correlated with the methine multiplet CH-33 (1.60, δ_C = 25.0) and the methylene carbon CH_2-32 (δ_H = 1.50, 1.36; δ_C = 41.5). The amide NH (5.99) coupled, *via* a H,H COSY correlation, with the α-methine CH-31 (δ = 4.64), and the latter showed in turn ^2J coupling (in HMBC) with the ester carbonyl C-36 (171.0).

From the observed NMR correlations (1D and 2D, Fig. 2), a pentacyclic triterpene parent skeleton was established for compound **1**, with a 3-β-acetoxy group in ring **A** along with the *gem*-dimethyls C-23 (δ_C = 28.0) and C-24 (δ_C = 16.6) at C-4 (δ_C = 38.0). The oxymethine proton H-3 showed a ^3J coupling to the acetoxy carbonyl C-38 (171.0), and the latter was further correlated (^2J) with the methyl singlet CH_3-39 (2.02). Moreover, ring **C** was established to bear an enone system between C-11 and C-13 (169.2), as the olefinic α-methine singlet H-12 (5.74) showed a ^2J coupling with the conjugated carbonyl C-11 (200.0). The latter (C-11) was connected with an sp^2-attached methine H-9 (s, 2.33) giving rise to a ^2J coupling in the HMBC experiment. On the other hand, β-H-18 (δ_H =

Table 3. ^{13}C and ^{1}H NMR data of dendrophen (**1**) (150/600 MHz, $CDCl_3$; *J* in Hz and multiplicities in parentheses).

Position	δ_C	δ_H
1	38.8	2.77 (dd, 13.6, 3.5)
2	23.5	1.66 (m), 1.58 (m)
3	80.6	4.49 (dd, 11.8, 4.7)
4	38.0	–
5	55.0	0.78 (m)
6	17.3	1.56 (m)
7	32.7	1.39 (m)
8	45.3	–
9	61.7	2.33 (s)
10	36.9	–
11	200.0	–
12	128.4	5.74 (s)
13	169.2	–
14	43.1	–
15	26.3	0.98 (m)
16	26.4	1.82 (m), 1.18 (m)
17	31.8	–
18	47.8	2.25 (dd, 12.0, 5.7)
19	41.8	1.76 (m)
20	43.6	–
21	37.3	1.36 (m)
22	31.3	1.97 (m)
23	28.0	0.85 (s)
24	16.6	0.85 (s)
25	16.3	1.13 (s)
26	18.4	1.10 (s)
27	23.2	1.34 (s)
28	28.4	0.78 (s)
29	29.3	1.12 (s)
30	175.6	–
30-NH	–	5.99 (d, 8.3)
31	50.4	4.64 (m)
32	41.5	1.50 (m), 1.36 (m)
33	25.0	1.60 (m)
34	21.7	0.91 (d, 6.0)
35	22.9	0.90 (d, 6.2)
36	173.5	–
37	52.3	3.71 (s)
38	171.0	–
39	21.3	2.02 (s)

2.25, δ_C = 47.8) was correlated to C-12 and C-13 by ^{3}J and ^{2}J connectivities, and additionally with C-17, 19, and 28, confirming the fusion between rings **C** and **E** *via* **D**. The remaining five methyl singlets (CH_3-23, 26, 27, 28, and 29) are located at C-10, C-8, C-14, C-17, and C-20, respectively, in the pentacyclic triterpene. This confirmed the basic skeleton of **1** as 3β-acetoxyolean-12-en-11-one (*i. e.* a β-amyrine system) [17–20] (**A** in Fig. 1). Finally, the amide carbonyl, C-30 (δ_C = 175.6), was fixed at C-20 due to the HMBC connectivities in ring **E**, as the methyl singlet CH_3-29 (δ_H = 1.12) displayed a crucial ^{3}J coupling towards C-20 and C-30.

Both partial structures **A** and **B** of dendrophen (**1**) were combined using the HMBC correlation between the amide NH (5.99) of the leucine (partial structure **B**) and the carbonyl C-30 (175.6) of the triterpenoid part **A** (Fig. 1). Thus, compound **1** was confirmed as 3-β-acetoxy-glycyrrhetyl-leucine methyl ester, which we named dendrophen.

The compound contains ten chiral centers, which were tentatively assigned by comparison of the triterpene parent skeleton with β-amyrine acetate [21] and glycyrrhetic acid [17], as well as by the high similarity of the optical rotation of the latter (α_D = +163°) with that of **1** (+165°). Consequently, dendrophen (**1**) has the same absolute configuration as glycyrrhetic acid, and in turn has an 18β instead of an 18α configuration.

The pentacyclic triterpenes constitute an important class of natural products, which are frequently isolated from plant sources. However, dendrophen (**1**) is the first example of a pentacyclic triterpene amino acid ester obtained from nature, although it was recently reported as a synthetic product. The available ^{1}H NMR data were identical, within the experimental error limits, to those of the natural product, however some assignments were different, and ^{13}C and 2D spectra were not reported [10].

Dendrotriol

Dendrotriol (**2**) was obtained as a colorless powder from fraction IV, after a series of chromatographic steps. The compound showed no UV absorption on TLC, behaving in a manner similar to compound **1**, however, it turned blue and later changed to brown on spraying with anisaldehyde/sulfuric acid, indicating the presence of a steroidal component. The molecular weight of **2** was established as 430 Da, and *via* HRESI MS, the molecular formula was established as $C_{28}H_{46}O_3$, bearing six double bond equivalents. In the NMR spectra, compound **2** showed signals for 6 methyls, 7 methylenes, 11 methines, and 4 quaternary carbons.

The ^{1}H NMR/HMQC spectra (Table 4) of **2** revealed two 2H multiplets at δ = 5.19 (144.9) and 5.09 (140.9, 124.7), representing three olefinic methines and one OH group (5.19). Two further OH signals (δ = 4.44 and 3.54) were found, along with two sp^3 oxymethines at δ = 3.78 (δ_C = 71.2) and 3.38 (δ_C = 77.4). Two methyl singlets and four methyl doublets were found in the region of δ = 0.99 – 0.55 (δ_C = 26.5 – 17.2), in addition to six sp^3 methines and seven methylenes. Based

Table 4. ^{13}C and ^{1}H NMR data of dendrotriol (**2**) (125/300 MHz, [D_6]DMSO; *J* in Hz and multiplicities in parentheses).

No.	δ_C	δ_H
1	37.7	1.29 (m)
2	26.5	1.00 (m), 1.44 (m)
3	71.2	3.78 (m)
3-OH		3.54 (s)
4	41.9	1.80 (m), 1.92 (m)
5	136.7	–
6	124.7	5.09 (brm)
7	77.4	3.38 (brm)
7-OH		4.44 (d, 5.7)
8	47.5	1.93 (m)
9	79.7	–
9-OH		5.19 (m)
10	47.6	–
11	27.8	1.40 (m)
12	48.2	1.98 (m)
13	44.2	–
14	59.4	1.80 (m)
15	26.5	1.00 (m), 1.44 (m)
16	26.5	1.00 (m), 1.44 (m)
17	60.5	1.30 (m)
18	17.2	0.55 (s)
19	22.9	0.91 (s)
20	45.4	1.80 (m)
21	26.5	0.99 (d, 6.6)
22	140.9	5.09 (brm)
23	144.9	5.19 (m)
24	47.5	1.93 (m)
25	36.4	1.62 (m)
26	25.2	0.82 (d, 6.6)
27	24.6	0.80 (d, 6.8)
28	23.1	0.89 (d,6.8)

on the ^{13}C NMR/HMQC data, the presence of further four quaternary carbons was established. Of these, one signal characterisitic of an olefinic bond was observed at δ = 136.7, while the remaining three were sp^3 hybridized: one oxygenated at δ = 79.7, and the other two at δ = 47.6 and 44.2.

According to the 2D correlations of **2** (Fig. 3), a 3β-hydroxy group is fixed in ring **A**, as shown by a ^3J HMBC cross signal between 3-OH (3.54) and the neighboring methylene carbon C-4 (41.9) and an H,H COSY coupling between CH_2-4 (1.92, 1.80) and the vicinal 3-oxymethine (3.78). The methyl singlet of CH_3-19 (0.91, δ_C = 22.9) at C-10 (δ_C = 47.6) displayed four crucial correlations with C-10, the olefinic quaternary atom C-5 (δ_C = 136.7), the oxygenated quaternary atom C-9 (δ_C = 79.7) and CH_2-1 (δ_C = 26.5), confirming a $\Delta^{5,6}$ double bond and a hydroxyl group at C-9. The latter showed further correlations with C-8 and C-10. The olefinic methine at C-6 (δ_H = 5.09) showed CH correlations with C-8 and C-10, and H,H COSY

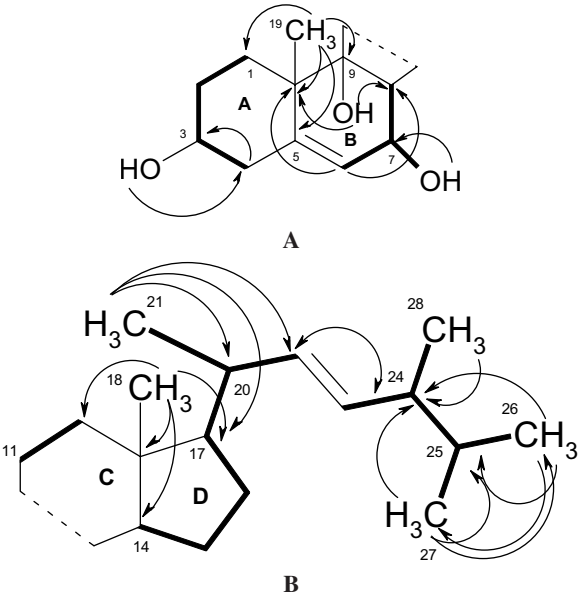

Fig. 3. Selected HMBC (→) and H,H COSY (▬, ↔) connectivities of two partial structures (**A**, **B**) in dendrotriol (**2**).

couplings with H-7 and H-8, thus completing ring **B**. According to the shift values (3.38, 77.4), C-7 is hydroxylated (7-OH, 4.44, Fig. 3).

In the HMBC experiment, the second methyl singlet (CH_3-18, 0.55, 17.7) showed four essential correlations towards CH_2-12 (δ_C = 48.2), C_q-13 (δ_C = 44.2), CH-14 (δ_C = 59.4), and CH-17 (δ_C = 60.7), joining the remaining two rings **C** and **D** *via* C-13 and C-14. The methyl doublet CH_3-21 (δ_H = 0.99, δ_C = 26.5) displayed three important correlations with CH-17 (60.7, ^3J), CH-20 (45.4, ^2J) and the olefinic CH-22 (140.9, ^3J), establishing the connection between ring **D** and the side chain *via* CH-17 (δ_H = 1.30) and CH-20 (δ_H = 1.80). The remaining complementary olefinic methine CH-23 (5.19) was assigned *via* an H,H COSY correlation to H-22 (5.09). CH-23 was in turn connected to a terminal isopentyl fragment, furnishing the second partial structure **B** (Fig. 3). The latter partial structure was extended by an H,H COSY correlation between CH_2-11 (δ_H = 1.40, δ_C = 27.8) and CH_2-12 (δ_H = 1.98, δ_C = 48.2). A further H-H coupling between CH-8 and CH-14 connected both fragments, resulting in structure **2** for dendrotriol (Fig. 4).

Expectedly, the EI mass spectrum of **2** did not show a parent molecular ion, however, fragment ions at m/z = 412 and 394 appeared as a result of the expulsion of two water molecules. This is indicative of the formation of a stable conjugated system, with ad-

Table 5. Antimicrobial assays of the soft coral *Dendronephthya hemprichi* varieties **I** and **II**.

Extract	*B. sub*	*E. coli*	*B. ser*	*Staph. aur*	*Sac. ser*	*Can. alb*	*Asp.niger*	*Botrytis sp.*	*Diplodia sp.*
I	7	–	9	7	7	7	7	–	–
II	7	–	11	7	6	6	6	–	–

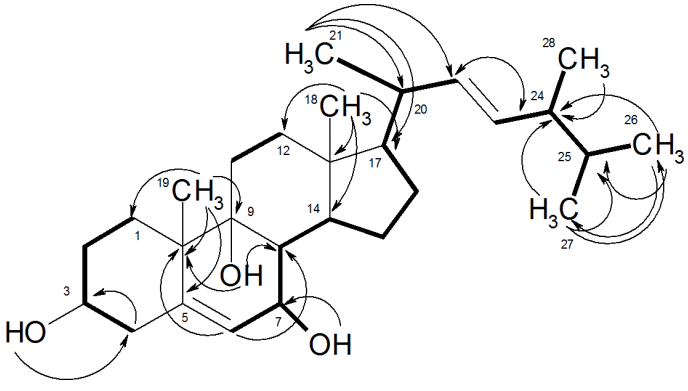

Fig. 4 Selected HMBC (→), and H,H COSY (▬, ↔) connectivities of dendrotriol (**2**).

ditional double bonds probably at the 7 and 9 (11) positions.

Biological activity

Based on agar diffusion tests of the crude extracts (100 μg per disc), the reddish orange [I] and reddish violet [II] varieties of the soft coral *Dendronephthya hemprichi* were examined against nine pathogenic microorganisms, belonging to Gram positive and negative bacteria, yeasts and fungi (Table 5). Both coral varieties exhibited similar activities, ranging from moderate to weak against most of the test organisms, except for *E. coli*, *Botrytis* sp. and *Diplodia* sp. The two pure compounds **1** and **2** were inactive against *Bacillus subtilis*, *Staphylococcus aureus*, *Streptomyces viridochromogenes* (Tü 57), *Escherichia coli, Candida albicans*, *Mucor miehi*, *Chlorella vulgaris*, *Chlorella sorokiniana*, *Scenedesmus subspicatus*, *Rhizoctonia solani*, and *Pythium ultimum* at a concentration of 40 μg per disc. Cytotoxic activity using the brine shrimp assay was also not found at 10 μg mL^{-1} for **1**, and was weak for **2** (mortality rate 4.8 %).

Experimental Section

Optical rotations: Polarimeter (Perkin-Elmer, model 343). The NMR spectra were measured on Varian Unity 300 (300.145 MHz) and Varian Inova 600 (150.820 MHz) spectrometers. ESI MS was recorded on a Finnigan LCQ with a quaternary pump Rheos 4000 (Flux Instrument). EI mass spectra were recorded on a Finnigan MAT 95 spectrometer (70 eV). GC-MS was measured on a Trace GC-MS Thermo Finnigan chromatograph, ionization mode EI (70 eV), equipped with a capillary column CP-Sil8 CB for amines (length: 30 m; inside diameter: 0.25 mm; outside diameter: 0.35 mm; film thickness: 0.25 μm). The analysis was carried out at a programmed temperature profile: initial temperature 40 °C (kept for 1 min), then increasing at a rate of 10 °C min^{-1} to the final temperature 280 °C (kept for 10 min); the injector temperature was 250 °C and the detector (mode of ionization: EI) temperature 250 °C; He was used as the 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). R_f values were measured on Polygram SIL G/UV$_{254}$ TLC cards (Macherey-Nagel & Co.). Size-exclusion chromatography was done on Sephadex LH-20 (Lipophilic Sephadex, Amersham Biosciences Ltd; purchased from Sigma-Aldrich Chemie, Steinheim, Germany).

Animal material

Two varieties of the soft coral *Dendronephthya hemprichi* (reddish orange [I], 1.92 kg, and reddish violet variety [II], 1.72 kg wet weight) were collected from the Red Sea about ∼ 30 km off the coast of Safaga, East Egypt, at a depth of ∼ 30 m, and stored in a freezer until extraction. The two varieties of *Dendronephthya hemprichi* were morphologically characterized by Dr. Mohamed Abd-Elghany, Hurgada, Egypt, and a specimen of each was deposited at Red Sea Marine Parks, P. O. Box 363, Hurgada, Red Sea, Egypt.

Prescreening

Small pieces (50 g) of the two raw organisms (I and II) were mechanically crushed separately, treated with DCM-MeOH (2 : 1) and kept at ∼ 5 °C for 8 d. After filtra-

tion, the DCM layers were extracted and evaporated to dryness, affording yellowish-brown crude extracts of 20 mg (Ia) and 40 mg (IIa). The remaining dry animals were re-macerated in ethanol; after filtration, the ethanolic extracts were concentrated *in vacuo*, and the obtained water residues were re-extracted with DCM, giving 60 mg (Ib) and 31 mg (IIb) as red crude extracts. According to biological (Table 5) and chemical (TLC) pre-screening results, both varieties of the coral *Dendronephthya hemprichi* were established to have identical activity and metabolic constituents.

Extraction and isolation

Based on the pre-screening results shown above, both the red and the yellow varieties of *Dendronephthya hemprichi* were combined, homogenized in a blender, macerated with 6 L of DCM-MeOH (2 : 1) and kept at ∼ 5 °C for 8 d. The solid material was filtered off, and the organic layer was evaporated *in vacuo* (extract I, 2.16 g).

The remaining animal material was re-extracted with 1.5 L ethanol, followed by concentration at 40 °C. The aqueous residue was re-extracted with 0.8 L dichloromethane, giving extract II (3.43 g). Both extracts I and II were combined to afford 5.6 g of a reddish-brown extract, which was in turn subjected to silica gel column chromatography (column 3 ×100 cm^2), eluting with a *n*-hexane-DCM-MeOH gradient (*n*-hexane 0.5 L, *n*-hexane-50 % DCM 0.25 L, DCM 0.5 L, DCM-1 % MeOH 0.2 L, DCM-2 % MeOH 0.2 L, DCM-5 % MeOH 0.5 L, DCM-10 % MeOH 0.5 L, MeOH 0.5 L). Based on TLC monitoring, visualized by UV and anisaldehyde/sulfuric acid spray, four fractions were obtained: FI (1.1 g), FII (1.9 g), FIII (0.5 g), and FIV (1.8 g). Fractions I, II were further fractionated to give three non-polar sub-fractions MSH3, MSH8 and MSH9, which contained 4-oxo-pentanoic acid according to GC/MS. Purification of fraction III was achieved on a silica gel column (2 × 50 cm^2), eluting with *n*-hexane-DCM, leading to fractions MSH11 and MSH12. GC-MS analysis of MSH11 indicated the presence of 2-octadecanone and juniper camphor (**4**). Fraction MSH12 was further purified on Sephadex LH-20 (DCM-MeOH, 6 : 4) and afforded dendrophen (**1**, 7 mg) as a colorless solid.

Further fractionation of the last fraction IV using silica gel (column 3 × 60 cm^2, DCM-MeOH) led to four sub-fractions IVa, IVb, IVc, and IVd. Purification of IVa on Sephadex-LH20 (MeOH) gave hexitol (46 mg) as a colorless powder. Purification of sub-fraction IVb on Sephadex LH-20 (DCM-MeOH, 6 : 4) afforded MSH6 and MSH7. GC-MS of MSH7 showed the existence of 2-methyl-acrylic acid 2-diethylaminoethyl ester (**3**). Re-purification of MSH6 by Sephadex LH-20 (DCM-MeOH, 6 : 4) yielded cholesterol (14 mg) as a colorless powder. Sub-fraction IVd was purified on silica gel (column 2 × 50 cm^2, DCM-MeOH), followed by Sephadex LH-20 (MeOH), resulting in dendrotriol (**2**, 8 mg) as a colorless solid.

Antimicrobial activity

Antimicrobial assays were conducted utilizing the disc-agar diffusion method [22]. The crude extracts were dissolved in $CHCl_3$ – 15 % MeOH (100 $\mu g\,mL^{-1}$), and paper disks with a diameter of 5 mm were soaked with the solution, dried in a laminar flow box and put on agar plates, inoculated with *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Saccharomyces cerevisiae*, *Candida albicans*, *Aspergillus niger*, *Botrytis* sp., and *Diplodia* sp. The plates were inoculated at 37 °C for bacteria (12 h) and at 27 °C for fungi (24 h), while the algal test strains were incubated at r. t. in daylight. The diameter of the inhibition zones was measured by a ruler. Compounds **1** and **2** 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 sterilized conditions.

Brine shrimp microwell cytotoxic assay

The cytotoxic assay was performed according to Takahashi *et al.* [23] and Sajid *et al.* [24].

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