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A SUCCINYLANTHRANILIC ACID ESTER AND OTHER BIOACTIVE CONSTITUENTS OF *JOLYNA LAMINARIOIDES*

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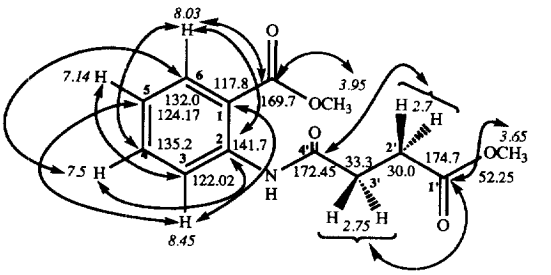
Key Word Index—*Jolyna laminarioides*; Scytosiphonaceae; brown alga; aromatic amide; antimicrobial compounds; chymotrypsin inhibition.

Abstract—Methyl 2-[propanamide-2′-methoxycarbonyl] benzoate, fucosterol, *trans*-phytol and *p*-formylphenol were isolated for the first time from a methanolic extract of *Jolyna laminarioides*. Methyl 2-[propanamide-2′-methoxycarbonyl]-benzoate exhibited chymotrypsin inhibitory activity and also found to be active against *Escherichia coli* and *Shigella boydii*. Fucosterol exhibited antifungal activity against *Curvularia lunata*, *Stachybotrys atra* and *Microsporum canis*. © 1997 Published by Elsevier Science Ltd

INTRODUCTION

Jolyna laminarioides Guimaraes [syn. *Endarachne binghamiae*] is a dark brown alga, first described by Agardh [1]. *Endarachne binghamiae* was initially described from Karachi by Nizamuddin and Farooqi [2] and later redesignated by Wynne and Banaimoon [3]. It was then placed under the synonym of *J. laminarioides* by Shaikh and Shameel [4]. So far little work has been done on the chemical constituents and the biological activities of this seaweed.



Scheme 1.

RESULTS AND DISCUSSION

The HREI mass spectrum of methyl 2-[propanamide-2′-methoxycarbonyl]-benzoate (**1**) showed the $[M]^+$ at *m/z* 265.0948, corresponding with the formula $C_{13}H_{15}NO_5$ (calcd. 265.0950), with seven degrees of unsaturation in the molecule. The mass spectrum showed a base peak at *m/z* 151 ($C_8H_9NO_2$), which indicated the presence of the fragment, methyl 2-aminobenzoate.

The UV spectrum of **1** exhibited absorptions at 222, 251 and 307 nm which indicated the presence of a conjugated aromatic system [5]. The IR spectrum exhibited strong bands at 3275 (N—H), 1720 (ester carbonyl) and 1685 (amide carbonyl) cm^{-1}.

Analysis of the ^1H NMR data of **1** indicated the presence of four aromatic protons, six methoxyl protons and four ethylene protons. The COSY spectrum exhibited vicinal couplings between H-6 (δ 8.03) and H-5 (δ 7.14), H-4 (δ 7.50) and H-5, H-4 and H-3 (δ 8.45) and H_2-2′ (δ 2.70) and H_2-3′ (δ 2.75). *Meta*-couplings between the H-3 and H-5 and between H-4 and H-6 were also observed in the spectrum.

The DEPT and BB (Broad-Band Decoupled) ^{13}C NMR spectra of **1** showed the resonances for all 13 carbon atoms in the molecule. The DEPT spectra (45, 90 and 135°) indicated the presence of four CH, two CH_2 and two CH_3 groups and by difference from the broad-band decoupled spectrum, five quaternary carbon atoms in the molecule. The structure was further confirmed by HMQC and HMBC (Scheme 1) techniques. The compound is thus the dimethyl ester of succinylanthranilic acid. The corresponding diethyl ester has been reported recently from another source [6].

The chymotrypsin inhibitory activity of **1** was also studied since a variety of diseases are associated with the excessive protease activity. Chymotrypsin, a serine

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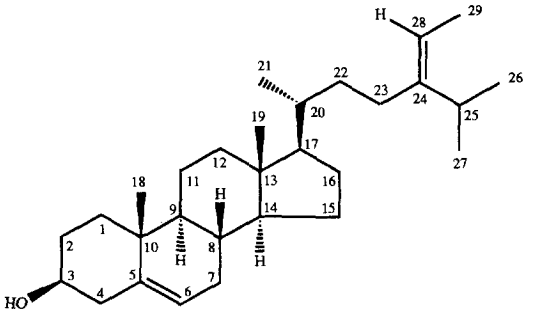

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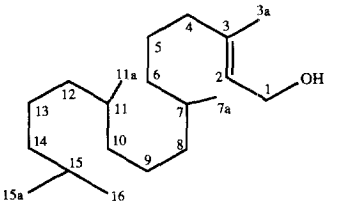

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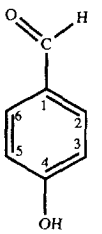

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protease, is involved in inflammatory situations in humans leading to destruction of fibrous proteins. Its excessive activity also leads to glomerulonephritis, pancreatitis, and other diseases. Its inhibition is therefore essential to control various disorders [7]. Compound **1** showed 42.54±0.89% inhibitory activity against chymotrypsin (Fig. 1).

The hexane fraction of the methanolic extract of *J. laminarioides* yielded the two known compounds, fucosterol (**2**) and *trans*-phytol (**3**), whereas the ethyl acetate fraction yielded *p*-formylphenol (**4**). Compounds **2**–**4** have not been previously reported from *J. laminarioides*. These compounds were identified by comparing their spectroscopic and other physical data with literature values [8–10].

EXPERIMENTAL

General. MS were recorded at 80 eV, IR as liquid films in $CHCl_3$ and UV in MeOH. ^1H and ^{13}C NMR were recorded at 400 and 180 MHz, respectively, in CD_3OD.

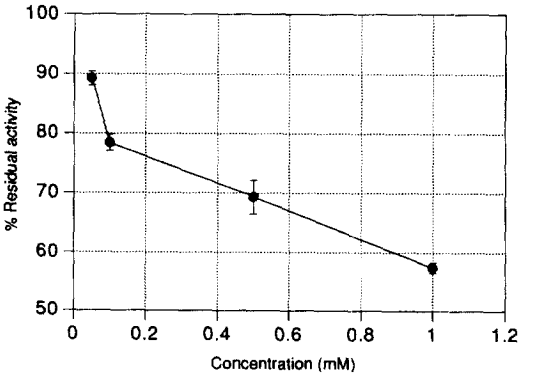

Fig. 1. Inhibition of chymotrypsin by compound **1**. Each value represents a mean ± s.e. (n = 3).

Plant material. About 9 kg (wet wt) of *J. laminarioides* was collected from mid and lower littoral rocks during August and September 1994 from the Buleji coast near Karachi city.

Extraction and isolation. Whole algae were washed with H_2O, air-dried for 3 days and then soaked in MeOH for one week. The MeOH extract was filtered, evap. under vacuum and the residue (180 g) was fractionated using hexane, $CHCl_3$, EtOAc and *n*-BuOH with H_2O. The extraction and isolation process was carried out under neutral conditions. The EtOAc fr. (4 g) was loaded onto a silica gel (500 g) column and subjected to gradient elution with hexane–$CHCl_3$ and $CHCl_3$–MeOH. The fr. obtained by elution with $CHCl_3$ was subjected to prep. TLC using $CHCl_3$ which yielded compound **1**.

Methyl 2-[*propanamide-2′-methoxycarbonyl*]-*benzoate* (**1**). Amorphous powder (20 mg, 0.02%), mp 50°. IR ν_{max} ($CHCl_3$): 3275, 1720, 1685 cm^{-1}. EIMS *m/z*: $[M]^+$ 265, 234, 202, 174, 151 (100%), 119, 55. HREIMS, *m/z* 265.0948 (calcd for $C_{13}H_{15}NO_5$, 265.0950), ^1H NMR (CD_3OD, 300 MHz): δ 8.03 (1H, *dd*, $J_{6,5}$ = 7.9 Hz, $J_{6,4}$ = 1.5 Hz, H-6), 7.50 (1H, *ddd*, $J_{4,5}$ = 8.5 Hz, $J_{4,3}$ = 7.3 Hz, $J_{4,6}$ = 1.7 Hz, H-4), 8.45 (1H, *dd*, $J_{3,4}$ = 8.4 Hz, $J_{3,5}$ = 1.9 Hz, H-3), 7.14 (1H, *dt*, $J_{5,6/5,4}$ = 8.4 Hz, H-5), 3.95 (3H, *s*), 3.65 (3H, *s*), 2.70 and 2.75 (4H, overlapping *m*, H_2-2′, H_2-3′).

Bioassays; (*i*) *Chymotrypsin inhibitory activity.* Chymotrypsin inhibitory activity of **1** was determined according to ref. [7]. Briefly, increasing concs of **1** (0.05 mM–1 mM) were incubated with chymotrypsin EC 3.4.21.1 (Sigma) in 50 mM Tris–HCl buffer (pH 7.6) at 25° for 30 min. After addition of substrate (*N*-succinyl-phenylalanine-*p*-nitroanilide, 1 mg ml^{-1}), the absorbance of liberated *p*-nitroaniline was measured at 410 nm. Inhibitory activity was calcd as the difference between the enzyme activity in the absence and presence of inhibitor. Results were compared with phenylmethylsulphonyl fluoride, a standard inhibitor of chymotrypsin.

(*ii*) *Anti-microbial activity.* Anti-fungal activity (Table 1) was determined by the agar tube dilution method, whereas antibacterial activity (Table 2) was determined by the agar well diffusion method [11, 12].

Table 1. MIC values of crude extract and compound **2** against pathogenic fungi

Fungi	Crude extract ($\mu g\ ml^{-1}$)	**2** ($\mu g\ ml^{-1}$)
Curvularia lunata	300	250
Epidermophyton floccosum	290	—
Stachybotrys atra	300	275
Drechslera rostrata	310	—
Allescheria boydii	280	—
Pleurelus ostreatus	350	—
Microsporum canis	325	250
Aspergillus niger	—	260
Trichophyton mentagrophytes	—	300

— = No activity.

Table 2. MIC values of crude extract, compounds **1** and **2** against pathogenic bacteria

Bacteria	Crude extract ($\mu g\ ml^{-1}$)	**1** ($\mu g\ ml^{-1}$)	**2** ($\mu g\ ml^{-1}$)
Streptococcus pyogenes	175	—	—
Corynebacterium diphtheriae	180	—	100
Shigella dysenteriae	170	—	—
Klebsiella pneumoniae	—	—	95
Escherichia coli	—	75	—
Shigella boydii	—	70	—
Staphylococcus aureus	—	95	—

— = No activity.

In the former, test tubes containing sterile Sabouraud dextrose agar were inoculated with different concentrations of stock solution of samples and kept in a slanting position at room temp. for solidification. Fungal cultures were inoculated on the slant and growth inhibition was observed after an incubation period of 7 days. For anti-bacterial activity one loop of 24 hr-old-culture containing *ca* 10^4–10^6 CFU (Colony Forming Units) was spread on the surface of Mueller–Hinton agar plates. Wells were cut in the medium with the help of a sterile metallic borer and 100 μl of each dilution was added to the respective wells. Zones of inhibition were measured after an incubation period of 24 hr. Nystatin and griseofulvin were used as standard anti-fungal antibiotics, whereas tobramycin and ampicillin were used as standard antibacterial antibiotics to compare the relative activity of samples. The MeOH extract of *J. laminarioides* showed promising anti-fungal and anti-bacterial activity against different pathogenic fungi and bacteria. This pronounced activity of the crude extract of *J. laminarioides* stimulated us to find out the compounds which are responsible for this broad spectrum of activity. Compounds **1** and **2** were tested for antimicrobial activity. The minimum inhibitory concn (MIC) values of these compounds against different fungi and bacteria were determined. *Escherichia coli* and *Shigella boydii* were found to be more sensitive to **1**. *Staphylococcus aureus* is also sensitive but at relatively higher concs of **1**. *Corynebacterium diphtheriae* and *Klebsiella pneumoniae* also showed some sensitivity to **2**. Compound **2** was also more active against the five fungi, *Curvularia lunata*, *Stachybotrys atra*, *Microsporum canis*, *Aspergillus niger* and *Trichophyton mentagrophyte*.

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