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PINNATIFOLIDE, A NEW METABOLITE FROM RED ALGA *LAURENCIA PINNATIFIDA* LAMOUR

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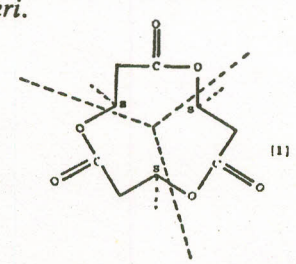
A new metabolite pinnatifolide has been isolated from methanolic extract of red alga *Laurencia pinnatifida*. It showed significant antibacterial activity against gram positive bacteria. Spectroscopic methods have been used to characterise pinnatifolide as tris-β-butyrolactone.

Key words: Pinnatifolide, Red alga, *Laurencia pinnatifida* Lamour.

Introduction

The red alga *Laurencia* has been subjected to intensive chemical investigation due to the presence of unusual halogenated sesquiterpenoid and C_{15} nonterpenoid structures [1]. The methanolic extract of *L. pinnatifida* has shown significant activity against gram positive bacteria [2].

We have already reported the isolation of pinnatazane [3], pinnatifidone [4], laurol [5], and now we wish to report the isolation of a new metabolite pinnatifolide (1) from methanolic extract of *L. pinnatifida* which to our knowledge has not been isolated so far from a natural source. It showed significant antibacterial activity against *S. dysenteria, S. sonnei, S. aureus* and *B. subtilis* and weak activity against *S. typhi* and *S. Schottmuelleri*.



[1]

(Dotted lines represent three units of trimer)

Experimental

Melting point was recorded in glass tube and was incorrected. I.R. spectrum was measured on JASCO IRA-1 spectrometer. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded in $CDCl_3$ on Bruker 400 and 100.61 Hz respectively. Mass spectrum was recorded on Finnigan MAT-112 and 312 double focussing mass spectrometer connected to PDP 11/34 computer system.

Collection and identification–The alga *L. pinnatifida* was collected from Buleji near Karachi coast of Arabian sea. It was identified by Prof. Mustafa Shameel, Department of Botany, University of Karachi, where a voucher specimen (no. MS-1734) has been deposited in the herbarium of the Botany Department.

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Extraction and isolation. 3 Kilogram (wet wt.) of *L. pinnatifida* was extracted in a Soxhlet apparatus with chloroform and then with methanol (each 8h). The methanolic extract after evaporation under reduced pressure was subjected to column chromatography on silica gel eluted with hexane, hexane:ether, ether, ether: chloroform, chloroform, chloroform:methanol and finally with pure methanol in order of increasing polarity. The compound (1) was eluted with pure chloroform.

m.p.: 252°, [α]D: -9.8° (c=0.02, chloroform); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ1.26 (3H, d, J=6.3 Hz), 2.40 (1H, dd, J=15.4, 5.8 Hz), 2.50 (1H, dd, J=15.4, 7.4 Hz), 5.25 (1H, m, $W_{1/2}$=9.8 Hz); ^{13}C-NMR ($CDCl_3$, 100.61 MHz): (ppm) 18.77 (CH_3), 40.79 (CH_2), 68.80 (CH), 169.09 (C=O); EIMS *m/z* 258 (M^+, trimer, $C_{12}H_{18}O_6$), 155 ($M^+ - C_4H_7O_3$), 86 ($C_4H_6O_2$, monomer).

Alkaline hydrolysis. 5 Milligram of compound (1) was refluxed with 10% methanolic KOH for 4 hrs. After evaporation of methanol under reduced pressure the residue was acidified with H_2SO_4 and then extracted with ethyl acetate which after evaporation afforded gummy compound, [α]D=+29° (c=0.341, $CHCl_3$) identified as 3-hydroxybutanoic acid.

Results and Discussion

The methanolic extract of *L. pinnatifida* was loaded on silica gel column which afforded crystalline compound (1) with pure chloroform and after recrystallization from methanol a white powder was obtained.

The ^{1}H-NMR spectrum exhibited signals at δ1.26 (3H, d, J=6.3 Hz), 2.40 (1H, dd, J=15.4, 5.8 Hz), 2.50 (1H, dd, J=15.4, 7.4 Hz) and 5.25 (1H, m, $W_{1/2}$=9.8 Hz). The irradiation at δ5.25 resulted in the simplification of signals at δ2.40 and 2.50 into two doublets (J=15.5 Hz) and (15.5 Hz) respectively. The methyl doublet at δ1.26 was changed into a singlet. While upon irradiation at δ2.40 and 2.50 the doublet at δ1.26 remained unchanged and the signal at δ5.25 was simplified into a distorted triplet. This clearly indicates the linkage of CH with

CH_2 and CH_3 groups while the CH_2 showed coupling with CH only.

The ^{13}C-NMR spectrum exhibited only four signals at 18.77 (CH_3), 40.79 (CH_2), 68.80 (CH), 169.09 (C=O) ppm. The chemical shift at 169.09 ppm indicates ester or carboxylic acid. The compound did not react with diazomethane or with acetic anhydride/pyridine showing the absence of carboxyl or hydroxyl group in the molecule and supporting the presence of a lactone ring. The alkaline hydrolysis of the lactone furnished 3-hydroxybutanoic acid, [α]D=+29° (*c*=0.341, $CHCl_3$), the structure of which is confirmed through ^{1}H-NMR spectrum. It exhibited a carbinylic signal which is shifted upfield to δ 4.21 as multiplet due to the presence of hydroxyl group instead of ester linkage while a multiplet appeared in the region between δ2.2-2.7 due to CH_2 group next to C=O function. The optical rotation of the compound is consistent with the S configuration of 3-hydroxybutanoic acid. The above spectroscopic data led to the deduction of the structure of compound as β-butyrolactone but the I.R. spectrum showed the carbonyl absorption at 1720cm^{-1} typical of an ester group instead of a four membered lactone. This was further supported by the mass spectrum of (**1**). It exhibited a very weak molecular ion peak at *m/z* 258 corresponding to the molecular formula $C_{12}H_{18}O_6$ which clearly indicates the presence of trimer of β-hydroxybutyric acid lactone. The other strong peak at *m/z* 155 appeared due to the loss of $C_4H_7O_3$ while the fragment at *m/z* 86 corresponds to the monomer of β-butyrolactone. On the above mentioned spectral and chemical evidence the structure (1) has been assigned to pinnatifolide.

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