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A New Halogenated Sesquiterpene Pinnatifidone from the Red Alga *Laurencia pinnatifida*

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From the ethyl acetate fraction of methanolic extract of red alga, *L. pinnatifida*, a new halogenated sesquiterpene named as pinnatifidone [1] has been isolated and the structure of this compound has been elucidated with the help of intensive spectroscopic studies.

Introduction

The red algae of the genus *Laurencia* have been investigated extensively for their chemical constituents due to the interesting metabolites present in them. During an antimicrobial screening study of Arabian marine algae [1] significant activity was displayed against *Bacillus subtilis*, *Bacillus megaterium*, and *Streptococcus viridans* by methanolic extract of *Laurencia pinnatifida*. We were therefore motivated to initiate an investigation of its chemical constituents. Previously we have reported a new halogenated sesquiterpene [2] from the chloroform extract and now we wish to report another new halogenated sesquiterpene, pinnatifidone [1] from the same source which is a new addition to the compounds having ether linkage between C-1 and C-10 as in the case of pacifinol [3].

The structure of compound **1** was elucidated on the basis of spectral data including extensive spin decoupling experiments and 2D-homo- and heteronuclear correlated NMR spectroscopy as well as n.O.e. difference measurements. The absolute configuration was decided with the aid of CD spectrum.

Results and Discussion

Unlike the crude extract [1] the pure compound **1** was found to be inactive against three gram-positive bacteria.

Pinnatifidone has the molecular formula $C_{15}H_{22}O_2BrCl$ on the basis of FD mass spectrum (M^+ at m/z 352, 350, 348). Its IR spectrum exhibited ab-

sorption band at 1700 cm^{-1} for carbonyl group. There was no absorption due to a hydroxyl group. Therefore, one oxygen in the molecule is probably present in the form of ether.

The ^{13}C NMR spectrum accounted for fifteen carbon atoms. One peak for ketonic carbon appeared at 212.46 ppm, two tertiary carbons bearing oxygen functionality in the form of ether exhibited peaks at 84.72, 79.50 ppm while those of a tertiary and a quaternary carbon bearing halogens appeared at 60.40 and 70.95 ppm respectively.

In the ^1H NMR spectrum a singlet at δ 1.80 is due to a methyl group attached to a carbon bearing a halogen atom. Two more methyl singlets appeared at δ 1.04 and 1.23 while the fourth methyl signal appeared as a doublet at δ 1.34. The downfield shift of this methyl could be due to the deshielding effect of the ketonic function next to this methyl group. The spectrum also showed an equatorial methine proton signal at δ 4.01 (t, $J = 3.4$ Hz), two axial methine protons at δ 4.14 (dd, $J = 12.8, 6.0$ Hz), 4.38 (dd, $J = 13.8, 7.0$ Hz).

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The coupling between various protons and the carbon proton correlation were established by homo- and heteronuclear 2D NMR spectroscopy (see Fig. 1 cosy-45 and Fig. 2 heterocopy).

Fig. 1. Cosy-45 spectrum of pinnatifidone.

The molecular formula, obtained from mass spectrum, as well as the signals in ^{13}C NMR and ^1H NMR spectra indicated that the compound **1** belongs to chamigrene class of sesquiterpene with an ether bridge between C-1 and C-10 as in the case of nidifocene [4]. The chemical shift of methyl present at C-3 in ^1H NMR of pinnatifidone is identical to nidifocene in which the positions of chlorine atom at C-3 and Br at C-4 have been placed ultimately after X-ray crystallography [5]. The positions of ketone at C-8 and methyl at C-7 have been assigned in pinnatifidone after homodecoupling experiment in ^1H NMR spectrum. It showed a quartet at δ 2.74 (1H, q, H-7) which after irradiation at δ 1.34 simplified into a singlet. This clearly indicated the presence of ketonic function at C-8 position and a methyl group at C-7 position. In the reverse case the signal of CH attached to CH_3 should give a multiplet instead of a quartet. The CH_2 group adjacent to $\text{C}=\text{O}$ group appeared at δ 2.58 (dd, $J = 10.0$, 5.1 Hz).

The 14-methyl group is apparently axial because on irradiation of the signal of this group, a 33%, 13.75% and 13.5% n.O.e. effect of the signals of H-7, H-12 and H-4 was observed. There was no n.O.e. effect on H-2 which would have been observed if 14-methyl group had been equatorial.

Fig. 2. Heterocopy spectrum of pinnatifidone.

Fig. 3. CD spectrum of pinnatifidone.

The absolute configuration of **1** has been elucidated from the CD spectrum (see Fig. 3). The magnitude of the cotton effect clearly rules out a twist conformation of the cyclohexanone ring. From the molecular models it can be seen that according to the octant rule, the circular dichroism within the $n-\pi^*$ transition of the carbonyl group will be positive regardless of the details of cyclohexanone conformation if the absolute configuration is that as given in structure **1**.

So in view of the above mentioned spectral data the structure of pinnatifidone has been assigned as **1**.

Experimental

The ^1H NMR and ^{13}C NMR spectra were recorded in CD_3OD at 300 MHz and 75.43 MHz respectively on Bruker AM-300.

Extraction and isolation

3 Kg (wet. weight) of *Laurencia pinnatifida* (Lamour) (herbarium No. MS-1734) was collected from the coast of Karachi and extracted in Soxhlet apparatus with chloroform and then with methanol. The methanolic extract was evaporated under reduced pressure. The residue thus obtained was partitioned between ethyl acetate and water. The ethyl acetate fraction after evaporation under reduced pressure afforded 28.3 g of crude extract which was subjected to silica gel column chromatography. The elution was carried out using solvent system hexane, hexane:benzene, benzene, benzene:ethyl acetate, ethyl acetate:methanol, and finally pure methanol.

The fraction eluted with pure benzene was again loaded on silica gel column which afforded 12 mg, semicrystalline compound **1**. It showed no activity against gram-positive bacteria.

$[\alpha]_D$: 36.03 ($C = 0.333$, MeOH).

IR (CHCl_3): 1700 cm^{-1} ($\text{C}=\text{O}$).

Mass (FD): M^+ at m/z 348, 350, 352 ($\text{C}_{15}\text{H}_{22}\text{O}_2\text{ClBr}$).

^1H NMR and ^{13}C NMR: See Table I and Discussion.

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Table I. ^1H and ^{13}C NMR Data of pinnatifidone.

Carbon	^{13}C NMR (CD_3OD , 75.43 MHz) (ppm)	Proton	^1H NMR (CD_3OD , 300 MHz) δ
1	84.72	1	4.01 (1H, t, $J = 3.4$ Hz)
2	43.64	2	2.50 (2H, dd, $J = 11.2, 2.76$ Hz)
3	70.95	3	—
4	60.40	4	4.38 (1H, dd, $J = 13.8, 7.0$ Hz)
5	35.83	5	1.90 (2H, dd, $J = 15.0, 14.9$ Hz)
6	44.00	6	—
7	45.8	7	2.74 (1H, q)
8	212.46	8	—
9	46.90	9	2.58 (2H, dd, $J = 10.0, 5.1$ Hz)
10	79.50	10	4.14 (1H, dd, $J = 12.8, 6.0$ Hz)
11	49.93	11	—
12	23.3	12	1.23 (3H, s)
13	21.26	13	1.04 (3H, s)
14	15.85	14	1.34 (3H, d, $J = 7.4$ Hz)
15	27.31	15	1.80 (3H, s)

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