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AN ANTIVIRAL SPHINGOSINE DERIVATIVE FROM THE GREEN ALGA *Ulva Fasciata*+

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ABSTRACT: A fraction showing *in vitro* and *in vivo* antiviral activity was isolated from the green alga *Ulva fasciata* found in the Indian Ocean off the West coast of India. The structure of its major component was established to be N-palmitoyl-2-amino-1,3,4,5-tetrahydroxyoctadecane on the basis of spectral studies.

Ulva fasciata, a green alga (Chlorophyta) belonging to the family *Ulvaceae* is of wide occurrence along the Malvan region of the West Coast of the Indian Peninsula. Most of the species of *Ulva* are used as food for humans and/or animals and as fertilizer for agriculture. Medicinal value for this alga has also been reported.[1]

In the course of an Indo-US collaborative project[2] on "Bioactive Substances from the Indian Ocean", samples of *Ulva fasciata* collected by scientists at the National Institute of Oceanography, Goa, India, were dried in the shade, powdered and extracted with ethyl alcohol. This alcohol extract showed antiviral activity against *Semeliki Forest Virus (SFV)* at 20 mg/mouse/7 days by giving 50% protection. We report here structural studies on the major component of this extract.

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Fractionation of the alcoholic extract[3] by repeated column chromatography over neutral alumina was monitored by antiviral tests.[4] Most of the activity was located in the EtOAc-MeOH fraction which yielded a solid material, m.p. 143-144°C , $[\alpha]_D$ + 8.2°(c, 0.5 MeOH) and was code named UF-131. This substance showed antiviral activity *in vitro* and *in vivo*.[4]

The IR spectrum of UF-131 indicated the presence of hydroxyl and amide groups. One of the products of the alkaline hydrolysis of UF-131 was identified as palmitic acid. Reaction of UF-131 with acetic anhydride and pyridine led to an acetate derivative, m.p. 66-67° C. The display of four additional methyl peaks in the ^1H NMR spectrum of this derivative showed it to be a tetra-acetate.

UF-131 was almost insoluble in the usual NMR solvents. Its tetra-acetate which was readily soluble in chloroform showed the following proton NMR spectrum ($CDCl_3$, 400 MHz):
δ : 6.5 (d, 1H, J = 10 Hz, N*H*-CO-), 5.12 (q, 1H, J = 4 Hz, 9 Hz), 5.10 (q, 1H, J = 10 Hz, 4 Hz), 4.95 (dt, 1H, J = 10 Hz, 3 Hz), 4.45 (m, 1H), 4.35 (dd, 1H, J = 12.5 Hz, 6 Hz), 4.0 (dd, 1H, J = 12.3 Hz, 3 Hz), 2.18 (s, 3H), 2.09 (s, 3H), 2.06 (s, 3H), 2.02 (s, 3H), 1.8 (t, 2H, J = 4.5 Hz), 1.25 (broad s), 0.9 (br t, 6H, J = 3 Hz).

The distorted shape of triplets at 0.9 ppm and a broad peak with large area at 1.25 ppm were indicative of the presence of long hydrocarbon chains. The four singlets at 2.02 - 2.18 ppm were assigned to the four acetyloxy groups while the signals at 4.0 - 6.5 ppm corresponded to hydrogens on five carbons bearing one amide and four acetoxyl substituents. The most down field signal (6.5 ppm) was easily assigned to the amide proton. Making this signal as the starting point, the sequence in which these substituents appeared on the carbon chain was determined by a set of decoupling experiments.

When the proton at 4.45 ppm was irradiated the following changes in the spectrum were observed:

(i) the quartets at 4.0 and 4.35 ppm became two doublets showing mutual coupling of 12.4 Hz (indicative of geminal coupling);

(ii) the signal at 4.95 ppm was unchanged;

(iii) the signal at 5.12 ppm was modified (quartet to doublet)

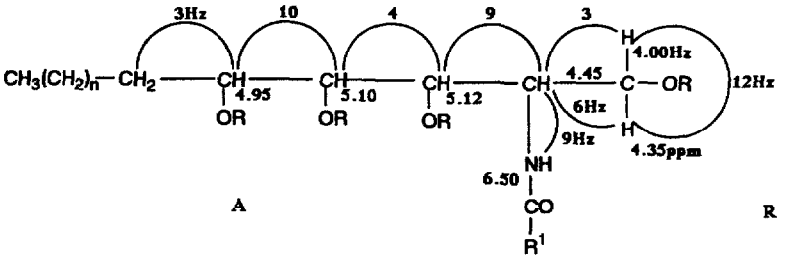
(iv) the doublet at 6.5 ppm became a singlet.

Upon irradiation of the proton at 4.95 ppm, the only change observed was the simplification of the quartet at 5.1 ppm to a doublet.

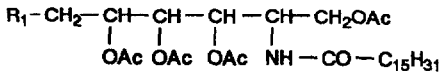
Several approximate coupling constants could be determined from the decoupled spectra (see **A**). Confirmatory structural evidence was obtained by matching the size of these coupling constants. Based on the above observations the partial structure **B** with specific juxtaposition of an amide and four acetoxy groups was deduced.

The structural features in **B** were in complete agreement with the ^{13}C NMR spectrum (62.21, t, C-1; 53.17, d, C-2; 72.59, d, C-3; 73.16, d, C-4/C-5; 76.97, d, C-5/C-4; -NH-*CO*- as a singlet at 175.35; four CH_3CO- singlets at 169.92 - 171.26 ppm).

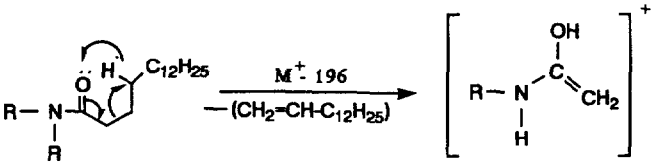
Extensive mass spectral studies were conducted on the tetra-acetate. The most significant information was obtained from FAB-MS which showed a pseudo-molecular (M+K)+ ion at m/z 778.5225 corresponding to the molecular formula of $C_{42}H_{77}NO_9$. The base peak at m/z 543 could be attributed to the ion **C** arising by a *McLafferty type rearrangement of the amide group. Combining this mass spectral information with the* chemical and NMR data, it was possible to deduce the structure (1) for UF-131. This compound is thus a sphingosine derivative.



A



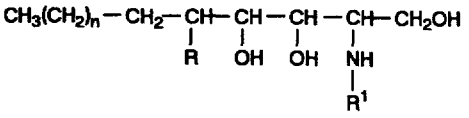
B



(M^+739 m/z)

m/z 543

C



(1) R = OH, R^1= $C_{15}H_{31}CO$, n = 11
(2) R = OH, R^1 = H, n = 11
(3) R = OH, R^1 = H, n = 13
(4) R = R^1 = H, n = 11
(5) R = R^1 = H, n = 13

A search of the literature showed that a 2-amino-1,3,4,5-tetrahydroxy-octadecane (2) and the corresponding eicosane (3) structures have been assigned to sphinganine (4,5-dihydrosphingosine) derivatives isolated from cerebrosides of bovine spinal cord and human brain by Prostenik et al.[5]

Other sphingosine derivatives - such as 2-amino-1,3,4-trihydroxy-octadecane and the corresponding eicosane, (4) and (5) - have been found in plant and animal tissues.[6,7] The 2S,3S,4R-configuration has been assigned to (4) on the basis of synthetic work by Mulzer and Brand.[8] The proton NMR spectra of the peracetates of (4) and UF-131 are fairly similar. Since the supply of UF-131 is now exhausted, stereochemical assignment will have to be determined by synthetic studies.

Previous workers[9] have reported the presence of an N-acyl-1,3-dihydroxy-4-octadecene and the corresponding dihydro derivative in marine alga of the *Caulerpa* species. The present report appears to be the first one to describe the occurrence of a 2-amido-1,3,4,5-tetrahydroxy-octadecane in a marine species. The biological role of these sphingosine derivatives in the marine world is as yet unknown.

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