Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Tetrahedron Letters, Vol.32, No.2, pp 169-172, 1991
Printed in Great Britain

0040-4039/91 $3.00 + .00
Pergamon Press plc

HALITUNAL, AN UNUSUAL DITERPENE ALDEHYDE FROM THE MARINE ALGA *HALIMEDA TUNA*

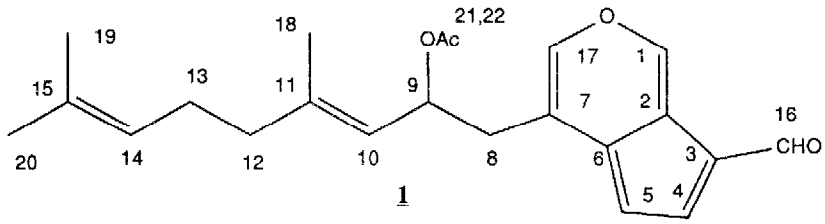
Frank E. Koehn*, Sarath P. Gunasekera, D. Nadine Niel and Sue S. Cross

Division of Biomedical Marine Research, Harbor Branch Oceanographic Institution, Inc.,5600 Old Dixie Highway, Ft. Pierce, Florida 34946 USA

Summary- Halitunal(**1**), a novel diterpene aldehyde possesing a unique cyclopentadieno[c]pyran ring system, has been isolated from the marine alga *Halimeda tuna*. The structure of **1** was elucidated by spectroscopic techniques. Halitunal shows antiviral activity against murine coronavirus A59 in-vitro.

Calcareous algae of the genus *Halimeda* have been the subject of a number of in-depth chemical investigations, due mainly to the fact that these organisms elaborate an array of biologically active terpenoid metabolites.[1-3] These studies have mainly focussed on the role played by these compounds in the chemical defense mechanisms of both the alga and its associated herbivores. [1,4] As part of our ongoing program to isolate new antiviral compounds from marine organisms we collected a sample of *Halimeda tuna*, the crude extract of which showed significant in-vitro activity against murine coronavirus strain A59.[5] We report here the isolation and structure elucidation of halitunal(**1**), the major antiviral constituent of this extract.

The sample of *Halimeda tuna* was collected by scuba near Chub Point in the Bahamas at a depth of 80 feet, and quickly frozen for transport. A 54 gram sample of thawed wet alga was extracted twice with 3:1 methanol:toluene and the extracts combined to give 131 mg of crude material after solvent removal. The crude solids were partitioned between water and n-butanol. The butanol fraction, which contained the antiviral activity, was chromatographed by reversed phase vacumn liquid chromatography (C_{18}, step gradient of 30%-100% CH_3CN:H_2O in 10% steps). Antiviral fractions were subsequently chromatographed by HPLC on silica gel in 25%-50% EtOAc:hexane or 5% isopropanol:hexane to give 5.6 mg of **1**.[6] (0.01% wet weight of alga, 4.3% of crude extract).



1

Halitunal was assigned a molecular formula of $C_{22}H_{26}O_4$ by HREIMS. The ^{13}C NMR spectrum showed 22 resonances which, together with a DEPT[7] experiment, showed seven sp^2 singlets, seven sp^2 doublets, of which one at δ 184.9 was assigned to the carbonyl of an α, β unsaturated aldehyde, one sp^3 doublet, three methylenes

and four methyl carbons. The ^{1}H NMR spectrum featured signals for an aldehyde proton at δ 9.96, six olefinic protons at δ 9.04 (s), 7.41 (d, J= 3.3 Hz), 6.68 (dd, J= 3.3, 0.8 Hz), 6.89 (s), 5.12 (dq, J= 9.1, 1.2 Hz),and 5.02 (m), benzylic protons at 2.88 (ddd, J= 13.7, 5.6, 0.6) and 2.43 (dd, J= 13.7, 7.7 Hz), a deshielded methine at 6.03 (ddd, J= 9.1, 7.7, 5.6 Hz), a pair of 2-proton methylene signals at δ 1.87 and 1.83, and four deshielded methyl groups. The results of a DQF-COSY experiment[8], a long range COSY experiment[9], an HMQC[10] experiment and an inspection of literature values previously reported for a *Halimeda* bis-nor diterpene acetate[2] clearly estabished the presence of partial structure A (Figure 1) in **1**.

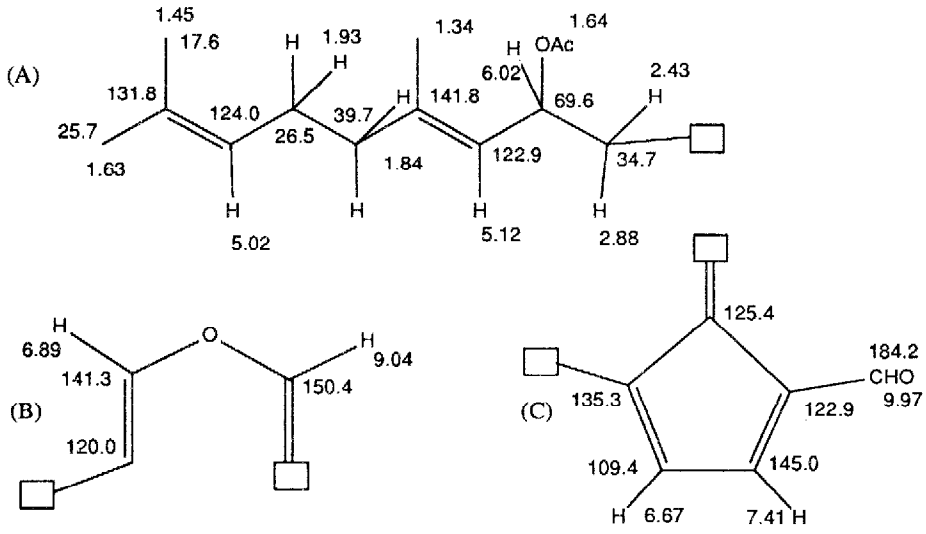


Figure 1. Partial structures and ^{1}H, ^{13}C NMR chemical shift values for **1**.

Aside from partial structure A, a total of seven degrees of unsaturation remain to be accounted for in the molecule, of which 5 are required by the remaining 8 olefinic carbon atoms and aldehyde group. Thus the remaining portion of the molecule must contain two rings. Due to the numerous remaining quaternary centers and limited amount of pure compound, we utilized an HMBC experiment[11] to establish the remaining connectivities. HMBC cross peaks were observed from H17 to C1, C6, C7, and from H1 to C17, C6 and C3. Carbons C1 and C17 must be attached to the remaining oxygen due to their chemical shifts and large one-bond heteronuclear J values (J_{CH}= 198 and 200 Hz for C1 and C17 respectively), and must both be adjacent to quaternary carbons since H1 and H17 are both singlets. Two and three-bond HMBC correlations allow placement of C7 adjacent to C17 and C6 adjacent to C7. A long range COSY experiment[9] showed a *W*-type H1-H17 coupling. These data are fully consistent with partial structure B in Figure 1.

The C16 aldehyde group was placed on C3 by virtue of a large HMBC correlation to H16, with an apparent two-bond CH coupling of *ca.* 24 Hz[12,13], and C2 was attached to C3 due to an HMBC correlation to C2 from the H16 aldehyde proton. HMBC correlations from H4 to C3, C16 , C5 and C6 along with correlations from H5

to C3, C4, C6, and C2 establish the cyclopentadiene partial structure C in Figure 1. A 3-bond HMBC correlation from H1 to C3 necessitates attachment of C1 to C2. A C7-C6 bond is indicated by a 3-bond HMBC correlation from H17 to C6, thus completing the fusion of partial structures B and C. The attachment of partial structure A at C7 can be made due to HMBC correlations from H8 to C7 (two bond), C17 and C6 (both three-bond). This completes the overall connectivity assignment, which is shown in Figure 2 with all HMBC correlations observed.

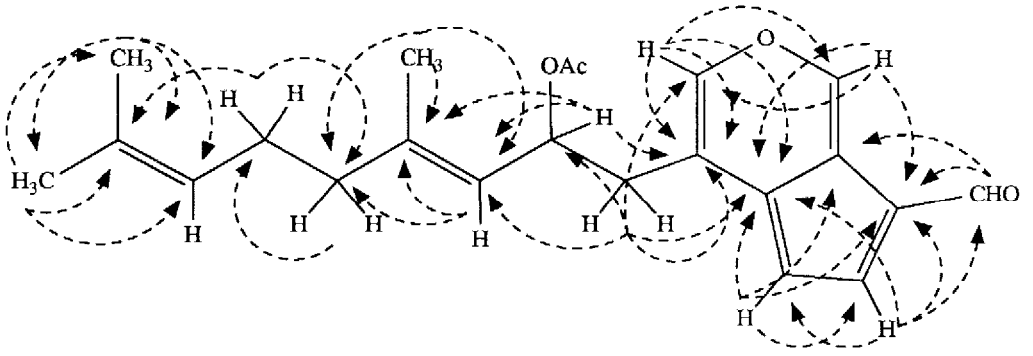


Figure 2. HMBC Correlations for **1**.

A straightforward biosynthetic pathway giving rise to **1** can be envisioned starting from the known diterpenoid tetra-acetate **2** , previously reported as a major metabolite of *Halimeda tuna*.[2] As shown in Figure 3, cyclization of enol acetate **2** results in an intermediate **3**, which, after loss of two molecules of acetic acid, rearrangement and hydrolysis, yields **1**.

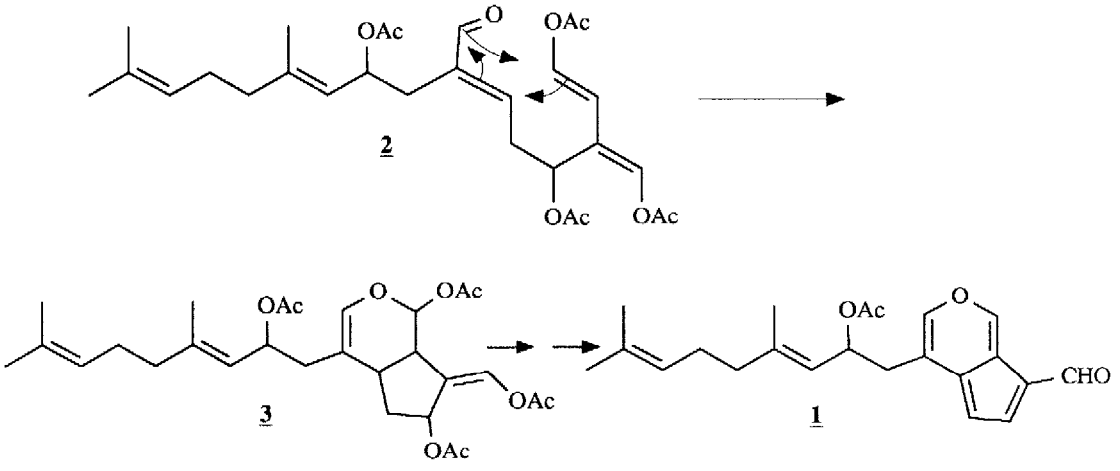


Figure 3. Proposed biosynthetic scheme for **1**.

Acknowledgements- We thank the University of Illinois, School of Chemical Sciences Mass Spectrometry Center for measurement of the mass spectra. This publication is Harbor Branch Oceanographic Institution, Inc. Contribution No. 809.

REFERENCES AND NOTES

1. W. Fenical and V. J. Paul, Science, 221, 747 (1983).
2. V.J. Paul and W. Fenical, Tetrahedron, 40(16), 3053 (1984).
3. L.M.V. Tillekeratne and F.J. Schmitz, Phytochemistry, 23(6), 1331 (1984).
4. V.J. Paul and K.L. Van Alstyne, J. Exp. Mar. Biol. Ecol., 119, 15 (1988).
5. Murine coronavirus strain A59 inhibitions were measured by reduction in cell fusion and cytopathic effects in NCTC 1469 mouse liver cells. Readings in these assays range from 0 for no observable inhibition of virus to 3+ for complete viral inhibition by the test compound. Halitunal(1) showed 2+ (ca. 50%) inhibition of viral replication at a dose of 20 µg per test well.
6. Halitunal(1): HREIMS *m/z* meas. 354.18228, 19.7%, calc. for $C_{20}H_{26}O_4$, Δ 0.8 mmu, UV (EtOH): λ_{max}= 426, 310, 287, 240, 227 nm ; ϵ = 4640, 3920, 6180, 7860, 8500; IR (microKBr) 1720, 1695, 1625, 1455, 1385, 1230, 1015 cm^{-1}; ^1H NMR (360 MHz, C_6D_6): δ 9.97 (1H, s, H16), 9.05 (s, 1H, H1), 7.41 (1H, d, J= 3.3 Hz, H4)), 6.89 (1H, s, H17), 6.68 (1H, dd, J= 3.3, 0.8 Hz, H5), 6.03 (1H, ddd, J= 9.1, 7.7, 5.6 Hz), 5.12 (1H, dq, J= 9.1, 1.2 Hz, H10), 5.02 (1H, m, H14), 2.88 (1H, ddd, 13.7, 5.6, 0.6 Hz, H8), 2.43 (1H, dd, J= 13.7, 7.7 Hz, H8), 1.87 (2H, m, H13), 1.83 (2H, m, H12), 1.64 (3H, s, H22), 1.63 (3H, d, J= 0.9 Hz, H20), 1.46 (3H, s, H19), 1.33 (3H, s, H18); ^{13}C NMR (90 MHz, C_6D_6): δ 184.2 (s, C16), 169.5 (s, C21), 150.4 (d, C1), 145.0 (d, C4), 141.8 (s, C11), 141.4 (d, C17), 131.8 (s, C15), 135.3 (s, C6), 125.4 (s, C2), 124.0 (d, C14), 122.9 (s, C3), 122.9 (d, C10), 120.2 (s, C7), 109.4 (d, C5), 69.6 (d, C9), 39.7 (t, C12), 34.7 (t, C8), 26.5 (t, C13), 25.7 (d, C20), 20.7 (q, C22), 17.6 (q, C19), 16.6 (C18).
7. M.R. Bendall, D.T. Pegg, D.M. Doddrell, D.H. Williams, J. Org. Chem., 47, 3021 (1982).
8. D. Marion and K. Wüthrich, Biochem. Biophys. Res. Comm., 113, 967 (1983).
9. A. Bax and R. Freeman, J. Magn. Res., 44, 542 (1981).
10. A. Bax and S. Subramanian, J. Magn. Res. 67, 565 1986..
11. A. Bax and M.F. Summers, J. Am. Chem. Soc. 108, 2093 (1986).
12. E. Breitmaier and W. Voelter, Carbon-13 NMR Spectroscopy, VCH, New York, 1987, pg. 141.
13. While apparent J_{CH} values in HMBC experiments are frequently inaccurate due to homonuclear coupling, nonabsorptive lineshapes and associated magnitude processing, in cases where there is no homonuclear coupling, large long range J_{CH} values (20 Hz) can be reliably identified. For a further discussion see: A. Bax and D. Marion, J. Magn. Res. 78, 186 (1988).

(Received in USA 1 October 1990)