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TETRACETYL CLIONAMIDE, A 6-BROMOTRYPTOPHAN DERIVATIVE FROM THE SPONGE *CLIONA CELATA*

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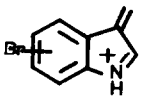
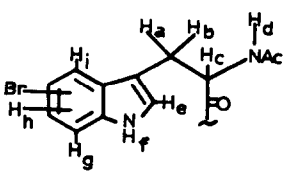
Ethanol extracts of the sponge *Cliona celata*, collected in British Columbia waters[1], show *in vitro* antibiotic activity against *Staphylococcus aureus*. Partitioning the concentrated ethanol extracts between ethyl acetate and water resulted in all the antibiotic activity residing in the organic layer. Silica gel chromatography of the ethyl acetate residues resulted in an extremely low total yield of material and failed to produce any active substances. LH20 chromatography followed by reverse phase HPLC has to date also failed to generate pure metabolites.

In order to circumvent the difficulties encountered in purifying the *Cliona* metabolites, the crude ethyl acetate extracts were acetylated at room temperature with acetic anhydride and sodium acetate. Silica gel chromatography of the acetylated crude material using methylene chloride, ethyl acetate gradients gave a number of pure compounds in high yield. The major component was tetracetyl clionamide (1), mp 209-211° (tetrahydrofuran, isopropyl ether), $[\alpha]_D$ + 45° (c .7, acetone), $C_{27}H_{26}N_3O_8Br$ (m/e 601.0889 - Br^{81}, calculated 601.0874), UV (MeOH, λ_{max} 227, ε 5.9×10^4, 290, ε 3.7×10^4). In the mass spectrum, 1 shows a parent ion doublet at 599,601 (1:1) indicative of one bromine atom, a major doublet at 540,542 (1:1) [M^+ - (NHAc + H)], and a base peak at 208,210 (1:1) [] which was immediately indicative of a bromo indole fragment.[2]

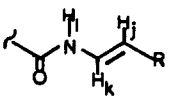
The infrared spectrum of 1 (KBr, 3418, 3280, 1760, 1627 cm^{-1}) along with the ^{13}C nmr spectrum [d_3-acetonitrile, δ 168.6 (1C), 169.3 (2C), 170.7 (1C), 171.2 (1C)] indicated that the molecule contains five carbonyl carbons, two of them equivalent. ^1H nmr studies (100 MHz, d_3-acetonitrile) further revealed that three of the carbonyls were phenol acetates (δ 2.23, s, 9H) and that one of the remaining carbonyls was an acetamide (δ 1.84, s, 3H).

The ^1H nmr spectrum also clearly shows that the bromo indole fragment in 1 is located in the following partial structure:



The side chain protons appear as a complex multiplet at δ 3.14 (H_a and H_b), a quartet at δ 4.59 (H_c, J = 7 Hz) and a doublet at δ 6.70 (H_d, J ≈ 8 Hz), signals typical of N-acetyltryptophan derivatives. Irradiation at δ 6.70 collapses the quartet at δ 4.59 into a triplet (J = 7 Hz) and irradiation at δ 4.59 collapses the doublet at δ 6.70 into a singlet and simplifies the multiplet at δ 3.14. The remaining protons in this fragment are found at δ 7.10 (H_e, s), 7.15 (H_h, q, J = 2 and 9 Hz), 7.51 (H_i, d, J = 9 Hz), 7.67 (H_g, d, J = 2 Hz) and 9.23 (H_f, bs).

A one proton doublet, found at δ 6.09 (H_j, J = 14 Hz) in the ^1H nmr spectrum, suggested that 1 contains a *trans* disubstituted olefin. Irradiation of this signal collapses a one proton quartet (H_k, J = 10 and 14 Hz) at δ 7.32 into a doublet (J = 10 Hz). Irradiation at δ 7.32 collapses the δ 6.09 signal to a singlet and also collapses a one proton doublet (H_l, J = 10 Hz) at δ 8.64 to a singlet. H_l exchanges in D_2O converting the δ 7.32 signal into a doublet (J = 14 Hz). This information suggested the partial structure:



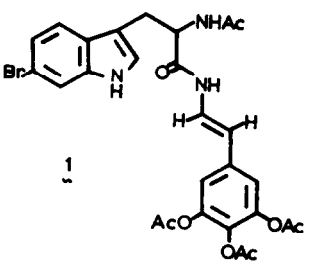
in which the carbonyl must be attached to the side chain of the tryptophan residue.

Ozonolysis of 1 (-78°, MeOH), followed by dimethylsulfide workup yielded 3,4,5-triacetoxybenzaldehyde (2) [IR (KBr) 1760, 1700; ^1H nmr (100 MHz, $CDCl_3$) δ 2.24 (s, 9H), 7.58 (s, 2H), 9.90 (s, 1H)] which was identical to a synthetic sample prepared from gallic acid. This established that the R group attached to the N-vinyl amide functionality was 3,4,5-triacetoxyphenyl and established the structure of 1 with the exception of the position of the bromine atom.

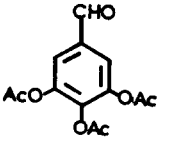
A second product obtained from the ozonolysis has IR, ^1H nmr and UV spectra consistent with structure 3. The thermal instability of this material has prevented us from obtaining a reliable mass spectrum so we cannot conclusively assign its structure.

Treatment of 1 with concentrated sulfuric acid-methanol (1:3) at room temperature provided a low yield (≈ 30%) of N-acetyl-6-bromotryptophan methyl ester (4). The aromatic region of the ^1H nmr spectra of 4 [(270 MHz, d_6 acetone) δ 7.16 (q, 1H, J = 8 and 1.5 Hz), 7.50 (d, 1Hz, J = 8 Hz), 7.58 (d, 1H, J = 1.5 Hz)] indicated that it contained the bromine atom in either the 5 or 6 position. To demonstrate that the bromine was indeed located on carbon 6, 4 was compared to authentic N-acetyl-5-bromo tryptophan methyl ester.[3] They differed in their ^1H nmr and infrared spectra, and they had differing HPLC retention times (Perkin Elmer-Silica A-hexane, isopropyl alcohol gradient).

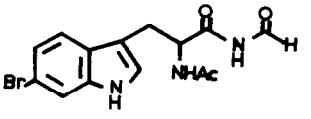
Hydrogenation of 1 (EtOH, 5% Pd/C) gave 5 as a single product. 5 has mass spectrum m/e M^+ 523.1970 calculated for $C_{27}H_{29}N_3O_8$, 523.1954; IR (KBr) 3400, 1760, 1635 cm^{-1}; ^1H nmr (100 MHz, d_3-acetonitrile) δ 1.81 (s, 3H), 2.25 (s, 9H), 2.64 (t, 2H, J = 7 Hz), 3.10 (m, 2H), 3.32 (q, 2H, J = 7 Hz), 4.48 (m, 1H), 6.54 (b, 2H), 6.88 (s, 2H), 7.0 → 7.7 (envelope, 5H), 9.1 (b, 1H). The two methylene groups in 5 resulting from hydrogenation of the N-vinyl amide olefin 1 appear as a triplet (J = 7 Hz) at δ 2.64 and a quartet (J = 7 Hz) at δ 3.32 in the ^1H nmr spectrum. D_2O exchange converts the quartet to a triplet (J = 7 Hz). These results confirm the


NHAc
Br
N
H
O
NH
H
H
AcO
OAc
OAc

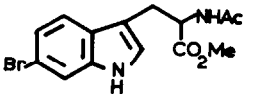
1


CHO
AcO
OAc
OAc

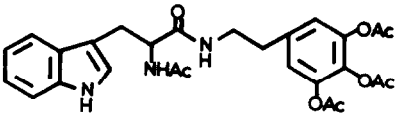
2


O
O
N
H
H
Br
N
H
NHAc

3


NHAc
Br
N
H
CO2Me

4


O
N
H
NHAc
N
H
OAc
OAc
OAc

5

presence of the N-vinyl amide functionality in 1.

Tetracetyl clionamide represents an additional example of a growing number of brominated indole derivatives found in marine organisms.[4] It is of particular chemical interest because it contains the rare N-vinyl amide functionality.

Cliona celata is often found boring into the calcium carbonate shells of *Balanus nubilus* and *Hinnites multirugosus*. The triphenolic portion of the underivatized clionamide should possess good calcium chelating properties suggesting a possible role for the compound in the sponge's boring capabilities.

ACKNOWLEDGMENTS

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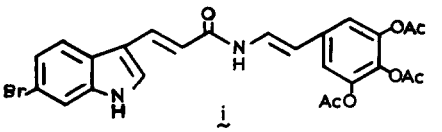
REFERENCES AND FOOTNOTES.

1. *Cliona celata* was collected at 20 metres' depth in Barkley Sound, Vancouver Island, and Horseshoe Bay. The author would like to thank Myriam Haylock of the Bamfield Marine Station and Dave Turpin, Institute of Oceanography, UBC, for assistance in collecting.

2. We encountered great difficulty in obtaining a mass spectrum of 1. Peaks containing more than one bromine atom were frequently encountered above m/e 599/601. We suspect that thermal dimerization of species such as i occurs on the probe.



i

See reference 4a for an example of similar difficulties.

3. Authentic 5-bromotryptophan was obtained from the Aldrich Chemical Company.

4. a) W.E. Raverty, R.H. Thomson and T.J. King, J.C.S. Perkin I, 1204 (1977); b) J.T. Baker and M.D. Sutherland, Tet. Letters, 43 (1968); c) G.E. Van Lear, G.O. Martin and W. Fulmar, Tet. Letters, 299 (1973); d) T. Kosuge, H. Zenda, A. Ochiae, N. Masaki, M. Noguchi, S. Kimura and H. Narita, Tet. Letters, 2545 (1972).