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Halitulin, A New Cytotoxic Alkaloid From the Marine Sponge *Haliclona tulearensis*

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Abstract: Halitulin (**2**), a novel bisquinolinylpyrrole has been isolated from the sponge *Haliclona tulearensis*. Its structure was elucidated mainly on the basis of spectroscopic data as well as chemical modifications. Halitulin was found to be cytotoxic against several tumor cells: P-388, A-549, HT-29 and MEL-28 in concentrations of 12-25 ng/ml.

In connection with our long-standing interest in the chemistry and bioactivity of marine sponges, we found that extracts of the Indo-Pacific sponge *Haliclona tulearensis* (class Demospongiae, order Haplosclerida, family Chalinidae, genus *Haliclona*), collected in Sodwana Bay, Durban, South Africa, were quite cytotoxic.

Many interesting N-containing metabolites were isolated from the genus *Haliclona*.[1-6] Recently, we reported the isolation of haliclorensin (**1**), a new N-(3'-aminopropyl)-3-methylazacyclodecane, from *H. tulearensis*.[7] The structure of the second N-containing metabolite from the same sponge designated halitulin, a substituted pyrrole, is the subject of this report. Over 250 pyrrole-containing compounds are known from marine organisms. A few that resemble the structure of halitulin are polycitone A, polycitrins A & B[8], the lamellarins[9] from ascidians and the storniamides[10] and arcyriarubins[11] from sponges.

Freshly collected *H. tulearensis* was frozen on site and kept frozen until needed. Freeze-dried sponge tissue (32g., dry wt) was extracted with methanol-EtOAc (1:1) to give a brown gum (2.9g) after evaporation. The latter extract was subsequently partitioned between aqueous methanol and CCl_4, $CHCl_3$ and n-butanol. The two latter fractions were individually fractionated by repeated chromatography on Sephadex LH-20 (eluting with $CHCl_3$:MeOH, 1:1) to afford halitulin (**2**, 180mg., 0.56% dry wt.).[12]

Halitulin (**2**) was analyzed for $C_{35}H_{40}N_4O_4$ by HREIMS (*m/z* 580.3054, 100%, Δmmu – 0.5) confirmed by positive and negative FABMS (*m/z* 581 and 579, respectively). The ^{13}C NMR spectrum (Table 1) showed, however, only 24 resonances - thirteen sp^3 carbons (one methyl, eleven methylenes and one methine) and eleven sp^2 carbons (five methines and six quaternary carbons) implying, therefore, the duplication of eleven carbon atoms. The duplicated part, according to the integration of the proton signals, was determined to be the aromatic portion of the molecule. Comparing the NMR data of the aliphatic part of **2** (Table 1) with haliclorensin (**1**),[7] together with the COSY, TOCSY and HMBC data, established their identity. The incorporation of **1** in halitulin was further supported by the two MS fragments at *m/z* 168 ($C_{11}H_{22}N^+$, 48%) and *m/z* 399 (MH^+-$C_{12}H_{24}N$, 90%), resulting from the preferable αβ to the aliphatic nitrogen-atoms' fragmentations at C-13,14 and C-12,13 respectively. Determination of the haliclorensin moiety in **2** left $C_{22}H_{14}N_3O_4$ (including the adjoining N-atom) with 17 degrees of unsaturation to be accounted for. Strong absorptions centred at 3200 cm^{-1} in the IR spectrum, the presence of four oxygen atoms in **2** and the absence of carbonyl and ethereal C-

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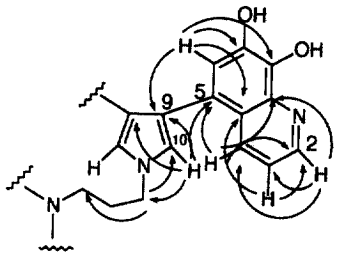
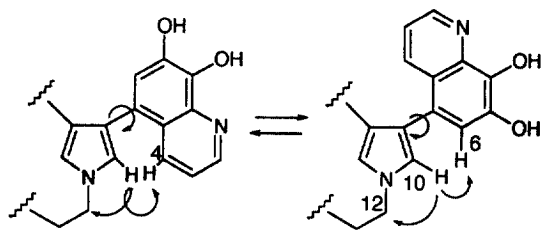


Key HMBC correlations of **2**



Key NOE's of **2**

atoms in the ^{13}C NMR spectrum suggested four OH groups. Indeed, acetylation of **2**, with a 1:1 mixture of Ac_2O/pyridine, overnight at r.t., afforded a very unstable phenol tetra-acetate (**3**), on the basis of the HREIMS which gave a suitable molecular ion and also four sequential losses of 42 m.u. and no loss of 60 m.u.[13] The four phenol acetate groups were in full agreement with the 1773 cm^{-1} IR absorption and the new methyls in the proton NMR. Furthermore, the ^1H-NMR spectrum, showing only two new signals at δ 2.37 and 2.50 ppm integrating for 6H each (in comparison with H_3-25), pointed clearly to symmetry in the aromatic part of **2**. The eleven sp^2 C-atoms, suggested six different double bonds, of which at least one has to be a C=N bond. A priori, more than one structure is possible, however, accounting for the above data, especially the 1D and 2D NMR spectra (Table 1), only one structure (discussed below) is possible, namely a bisquinolinylpyrrole. Two of the double bonds, carrying H-2 (δ 8.56) and H-10 (δ 7.04), with $^1J_{CH}$ values of 179 and 184 Hz, respectively, have to be adjacent to N-atoms, and moreover to be part of a quinoline and a pyrrole.[14] The ^1H and ^{13}C chemical shifts of the aromatic part (Table 1) implied a substituted quinoline system. Furthermore, the three proton spin system, confirmed by a COSY experiment, [δ 8.56 d ($J_{2,3}$ = 4.9 Hz), 7.20 dd (J = 4.9 and 8.3 Hz) and 8.51 d ($J_{3,4}$ = 8.3 Hz)] indicated, according to the 4.9 Hz coupling constant characteristic for a coupling constant next to a nitrogen atom, that the pyridine ring of the system is free of substitution. On the other hand, the adjacent benzene ring, carrying a single proton (δ 7.28 brs), has to be tri-substituted. That is, one position being the linkage to the rest of the molecule and the two others bearing OH groups. Empirical calculations of the carbon chemical shifts,[15] agreed best with a 5-substituted-7,8-dihydroxyquinoline, a suggestion that was confirmed in two ways: a. reaction of halitulin with $NaIO_4$ (known to oxidize catechols to o-quinones)[16] in a 1:1 mixture of EtOH:H_2O, afforded, on the basis of the change in the UV spectrum[17] and change in color from orange to red, an o-quinone and b. from the measured NOE's, *vide infra*. All the above data suggested a 3,4-bisquinolinylpyrrole, which is in full agreement with the results from the HMBC experiment. To distinguish between the very close shifts of C-4a, 6 and 10, a 1D INAPT experiment was undertaken.[18] Two key NOE's, that supported unequivocally the suggested structure, were between H-6 and H-10 and between H-4 and H-10. Both NOE's being possible due to rotation around the C-5,9 bond, and only possible for the suggested isomer.[19]

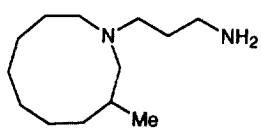
Halitulin, to the best of our knowledge, is the first natural compound to be discovered that embodies a 7,8-dihydroxyquinoline system. A very few other dihydroxyquinoline-containing compounds are known e.g. luzopeptin, a terrestrial Actinomadura antimicrobial metabolite[20] and the marine sponge *Verongia aerophoba* metabolite 3,4-dihydroxyquinoline-2-carboxylic acid.[21]

Compound **2** is sensitive to light and air, conditions under which, most likely, the azacyclodecane-nitrogen oxidizes. This is seen from the appearance of a weak peak at *m/z* M+16 in the mass spectrum. As a result, two N-oxide isomers are obtained causing the appearance of two new doublet methyl resonances (of Me-25) in the NMR spectrum. Subsequently, the dihydroxy quinoline also oxidizes to the o-quinolinoquinone.

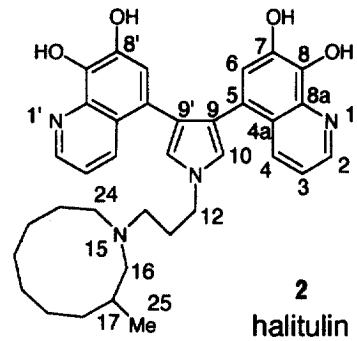
The structure of halitulin, as far as the aromatic part is concerned, resembles the polycitone[1] and the lamellarins.[2] However, the two phenyl-C_3 units of the latter's biogentic precursors, are suggested (Scheme) in

this case, to be replaced by two 5-substituted-quinoline-C_3 compounds which subsequently will undergo decarboxylation. There does not seem to be a simple way to suggest the biogenesis of the latter C_9N-C_3 unit. The haliclorensin (1) biogenesis (replacing the phenethylamine, as suggested earlier, for the above compounds[7]) is similar to the one suggested for manzamine C.[22]

Halitulin (2) was found to have cytotoxic activity. The activity, IC_{50} values, against cell cultures of P-388 murine leukemia, A-549 human lung carcinoma, HT-29 human colon carcinoma and MEL-28 human melanoma is 0.025, 0.012, 0.012 and 0.025 μg/ml respectively.



1
halichlorensin

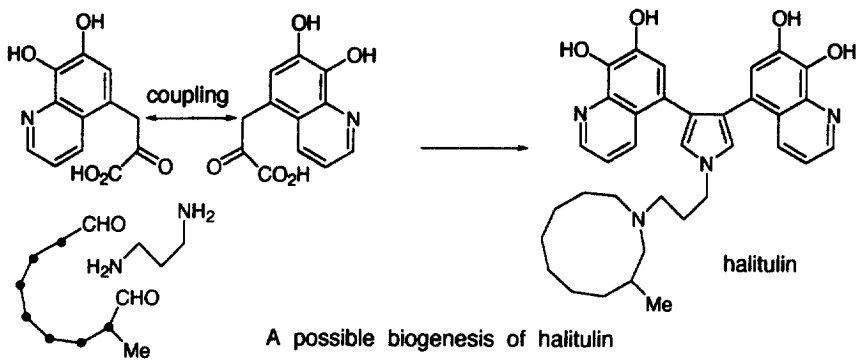


2
halitulin

Table 1. ^1H(500 MHz) and ^{13}C(125 MHz) NMR data of halitulin (2) in $CDCl_3$.

No.	δ_C(m)	δ_H (m, *J* in Hz)	HMBC (H to C)	No.	δ_C(m)	δ_H (m, *J* in Hz)
2	145.1 d	8.56 (d, 4.9)	3, 4, 8a	13	26.1 t	2.56 (m)
3	117.1 d	7.20 (dd, 8.3, 4.9)	2,4a	14	54.4 t	3.23 (m)
4	141.6 d	8.51 (d, 8.3)	2, 5, 8a	16	57.7 t	3.27 (m), 2.97 (m)
4a	122.9 s			17	28.3 d	2.32 (m)
5	126.7 s			18	32.7 t	1.60 (m)
6	123.2 d	7.28 brs	4a, 7, 8, 9	19	24.4 t[a]	1.36-1.62 (m)
7	148.1 s			20	24.2 t[a]	1.36-1.62 (m)
8	131.2 s			21	23.9 t[a]	1.36-1.62 (m)
8a	130.1 s			22	23.6 t[a]	1.36-1.62 (m)
9	118.8 s			23	22.0 t	1.96 (m)
10	122.6 d	7.04 brs	5, 9, 12	24	50.9 t	3.40 (m), 3.75 (m)
12	46.8 t	4.23 (t, 6.3)	10, 13, 14	25	20.7 q	1.09 (brs)

[a] exchangable [b] The chemical shifts are strongly influenced by concentration and pH e.g. C-4a, 6 and 10 resonated in another experiment at 122.5, 122.5 and 122.1 respectively. [c] The following $^1J_{CH}$-values have been measured for C-2, 3 ,4 ,6 & 10: 179, 166, 161, 160 and 184 Hz respectively. [d] Besides the COSY correlations of H-2 – 4, the aliphatic protons gave the expected COSY, TOCSY and HMBC correlations as detailed for haliclorensin earlier.[7] [e] INAPT correlations were found between (H to C): 2/8a, 3/4a, 4/5,8a, 6/4a,8,9 and 10/9.



A possible biogenesis of halitulin

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12. **2**, orange foaming oil; $[\alpha]_D$ +7.5 (c 2.8, MeOH), ν_{max} 3000-3400, 1623, 1597 cm^{-1}, λ_{max} (MeOH) 212(29200), 252(31600), 364(4400), λ_{max} (MeOH–OH^-) 214(24700), 264(14800), 350(4650).
13. **3**; EIMS *m/z* 748(5%), 706(50), 664(100), 622(100), 580(48), 525(10), 483(25), 441(42), 399(98), 168(37), HREI 664.3261(Δmmu – 0.1), 706.3382(Δmmu 1.1), 399.1220(Δmmu – 0.1). δ_H (*J* in Hz) 0.90 (d, 3H, *J* = 6), 1.51 (brs, 5H), 1.61-1.69 (m. 10H), 1.86 (m, 1H), 1.97 (m, 1H), 2.22 (m, 1H), 2.24 (m, 1H), 2.37 (brs, 6H), 2.50 (brs, 6H), 2.65 (m, 1H), 2.84 (brt, 1H), 4.16 (m, 2H), 7.03 (brs, 2H), 7.15 (dd, 2H, *J* = 8, 4), 7.28 (brs, 2H), 8.23 (d, 2H, *J* = 8.6), 8.76 (d, 2H, *J* = 2); δ_C 19.6, 20.6q x2, 20.7q x2, 22.3t, 24.5t, 24.7t, 26.0t, 26.5t, 29.0t, 30.2t, 31.9t, 48.5t, 52.3t, 53.5t, 60.8t, 120.6s, 120.7d, 121.7d, 123.4d, 125.9s, 132.4s, 134.6d, 136.4s, 141.7s, 142.1s, 150.5d, 168.1s, 168.5s.
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