# TUBASTRAINE: ISOLATION AND STRUCTURE OF A NOVEL ALKALOID FROM THE STONY CORAL TUBASTRAEA MICRANTHA[†]

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Abstract The non-symbiotic marine coral Tubastraea micrantha was found to contain a novel alkaloid along with a previously known sesterterpene. The structures of the isolated metabolites were determined by spectroscopic methods.

The non-symbiotic stony coral Tubastraea micranthahas been reported to be avoided by the Crown-of-Thorn seastar--Acanthaster planci--the major predator of stony corals in the Pacific.[1] This observation prompted us to investigate the secondary metabolites of this coral with special emphasis on the isolation of novel compounds followed by biological evaluation of the isolated metabolites. An examination of the ethanol extract of T. micrantha has resulted in the isolation and identification of a number of anthraquinoid derivatives[2] which we have speculated could be responsible for the avoidance response. In this communication we report the structure of a novel chromone alkaloid-tubastraine (I), along with the isolation of a known sesterterpene--heteronemin (II)[3] from this coral.

The residue from the ethanol extract of the air dried coral was defatted by triturating with hexane, followed by extraction with $CHCl_3$. The residue from the $CHCl_3$ extract, upon repeated chromatography on RP-8 columns, gave a glassy compound (I) mp 122°C, ir: 3400,(OH) 1735, 1730 (C=O ester) and 1660 (C=O), 1612 and 1560 (γ-pyrone). The $^1$H-nmr spectrum, in $CDCl_3$ indicated the presence of a hydrogen-bonded hydroxyl (δ 12.71, slowly exchanged upon shaking with $D_2O$) along with the presence of two disubstituted aromatic nuclei [δ 7.96 (2H,dd,J = 8.3,2.2Hz), 7.81(2H, dd,

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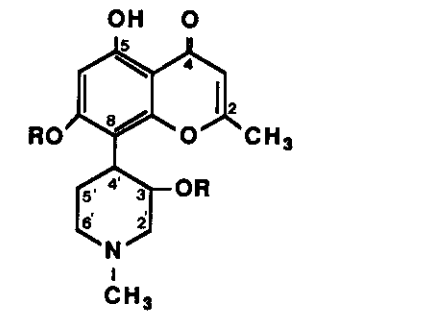
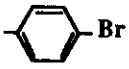
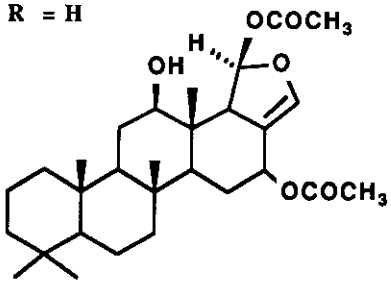



I. R = CO-  -Br

III. R = H




II

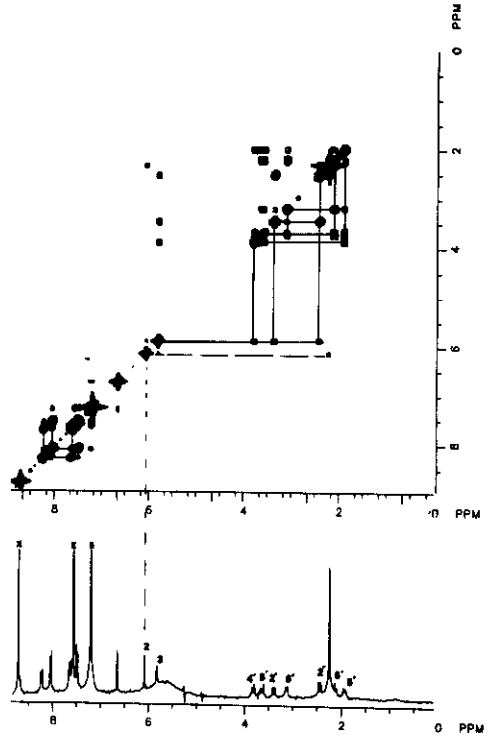



Figure 1: Symmetrized autocorrelated homonuclear (COSY) NMR spectrum of tubastraine in deuteriopyridine taken at 300.068 MHz. The normal spectrum (diagonal) is presented as a convensional high resolution spectrum (below) coupled resonances correlated via the off-diagonal responses, for which partial connectivity pathways are shown.

J = 8.3,2.2Hz), 7.67 (2H, dd, J = 8.3,2.2 Hz) and 7.46 (2H, dd, J = 8.3,2.2Hz). Protons at δ 7.96 and 7.46 and 7.81 and 7.67 were mutually coupled, as determined from a $^{1}H$-$^{1}H$ correlation (COSY) spectrum], two aromatic/vinylic protons [δ 6.50 (1H, s) and 6.08 (1H, d, J = 0.4Hz)], two methyls [δ 2.38 (3H, s) and 2.27 (3H, d, J = 0.4Hz)] and a proton (δ 5.60) on an oxygenated carbon. One of the two methyls (δ 2.27) was coupled to the proton resonating at δ 6.08 (as determined from a COSY spectrum). The $^{1}H$-nmr in pyridine showed the presence of seven protons resonating and at δ 3.81, 3.64, 3.38, 3.12, 2.43, 2.09 and 1.96. The $^{1}H$-$^{1}H$ COSY spectrum in pyridine (Fig I) showed that the methine proton (δ 5.60) was coupled to three protons (δ 3.81, 3.38 and 2.43). The protons at δ 3.38 and 2.43 were mutually coupled and connectivity stopped there. The proton at

δ 3.81 was coupled to two protons resonating at δ 3.64 and 1.96. The proton at δ 3.64 was, in turn, coupled to protons at δ 2.09 and 1.96 which were mutually coupled. The connectivities of these protons along with the presence of three carbons [56.4(t), 54.2(t), and 44.4(a) ppm] attached to a nitrogen(s), in the $^{13}$C-nmr spectrum, suggested the presence of a N-methyl-3,4-disubstituted piperidine moiety in I. A $^{1}$H-$^{13}$C COSY spectrum was used to identify the specific proton resonances associated with each individual protonated carbon. The $^{13}$C-nmr spectrum of I showed the presence of 30 carbons [13 x C, 12 x CH, 3 x $CH_2$ and 2 x $CH_3$]. In analogy with the literature[4] the $^{13}$C-nmr suggested the presence of a chromone nucleus [182.4(C4), 165.8(C7), 163.1 (C-10), 160.9(C5), 156.5(C2), 108.5(C6), 104.9(C9), 103.8(C8) and 100.2(C3)ppm] with possible substitutions at C2, C5, C7 and C8. The presence of 12 aromatic carbons along with two carbonyls (165.43ppm, 2C) in the $^{13}$C-nmr spectrum coupled with the connectivities of aromatic protons supports the presence of two p-bromobenzoic acid residues in I. One of the two p-bromobenzoic acid residues could be safely assigned to C3 of the piperidine moiety to satisfy the resonance of the methine proton at δ 5.60. Since the chromone nucleus has only two protons (assigned to C3 and C6, on the basis of literature values[5]), the second p-bromobenzoic acid residue must be attached to C7 leaving the piperidine moiety to be fixed at C8. This assignment is supported by the position of C10 in the $^{13}$C nmr spectrum of I.[4,6] The low resolution mass spectrum of I gave the highest peak at m/z 487 and 489. The presence of a p-bromobenzoic acid in I was also evident from the fragments at m/z 287, (487 - $C_7H_5O_2Br$) 200 ($C_7H_5O_2Br$) 202, 183 ($C_7H_4OBr$), 185, and 155 ($C_6H_4Br$), and 157. The structure was finally confirmed by the conversion of rohitukine[6] (III, a secondary metabolite of the plant - Amoora rohituka) into tubastraine. Compound II was isolated from the earlier fractions of the LH-20 column chromatography of the chloroform extract and was identified as heteronemin on the basis of $^{1}$H and $^{13}$C nmr, ms and interpretation of the $^{1}$H-$^{1}$H and $^{1}$H-$^{13}$C COSY spectral data.[7]

T. micrantha has been reported[2] to have an unusually high concentration of benzoic acid which has not been encountered before in marine invertebrates, however benzoic acid derivatives have been isolated from the sea grass - Posidonia oceanica[8] as well as from the cultures of chromobacterium sp.[9] Although flavonoids and chromones are common plants metabolites, only favonoids have been encountered in marine invertebrates. Tubastraine thus represent the first example of a chromone containing metabolite of a marine invertebrate. Heteronemin has been isolated from a number of sponges inhabiting the south Pacific, this is the first report on the isolation of II from a coral. Since T. micrantha does not harbor symbionts, it is quite possible that the isolated compounds could have been derived from dietary sources.

EXPERIMENTAL

Mp were recorded on a Fisher-Johns apparatus. Spectra were recorded on the following instruments: Ir: Perkin-Elmer model 283; mass spectra: Finnigan model 1020 equipped with an Incos Data System. Nmr spectra were recorded on a Nicolet NT300 wide bore spectrometer operating at 300.068 and 75.457 Hz for $^{1}H$ and $^{13}C$ observation, respectively. The instrument was controlled by a model 29C pulse programmer and was equipped with a 5mm $^{1}H/^{13}C$ dual tuned probe. Both $^{1}H$-$^{1}H$ and $^{1}H$-$^{13}C$ correlation spectra were recorded as described elsewhere.[10]

An air dried sample (50g) of T. micrantha, collected from the waters of Palau in summer 1983, was extracted with EtOH and the extract was evaporated to dryness to yield a residue (3.99g). The residue after defatting with hexane was extracted with $CHCl_3$. The residue from the $CHCl_3$ extract (1.7g) was chromatographed on a LH-20 column (2.5 x 100 cm), which was eluted with 10% MeOH in $CH_2Cl_2$. Fractions containing Dragendorff's positive spots (21-28, 8 ml each) were combined and rechromatographed on a RP-8 column (0.5 x 60 cm). Elution of the column with 5% MeOH in $CHCl_3$ gave a group of fractions (5-8, 4 ml each) which upon rechromatography and evaporation gave 6.5 mg of I as glassy residue. Mp 122°C, $^{1}H$ nmr ($CDCl_3$): δ 12.71(bs), 7.960 (2H, dd, J=8.3,2.2Hz), 7.818 (2H, dd, J=8.3,2.2Hz), 7.670 (2H dd, J=8.3,2.2Hz), 7.469 (2H, dd, J=8.3,2.2Hz), 6.506 (1H,s), 6.083 (1H,d,J=0.4Hz), 5.606 (1H, d, J=2.2Hz), 3.802, 3.573 (5H,m), 3.056 (1H,J=12.5 Hz), 2.738 (3H,s), 2.271 (3H,d,J=0.4Hz), and 1.905 (1H d, J=12.5Hz). $^{1}H$-Nmr ($C_5D_5N$): δ 13.72 (1H,bs), 8.224 (2H, d, J=8.2Hz), 8.034 (2H, d, J=8.4Hz), 7.635 (2H, d, J=8.4Hz), 7.484 (2H, d, J=8.4Hz), 6.647 (1H,s, 6-H), 6.073 (1H, s, 3-H), 5.816 (1H, s, 3'-H), 3.819 (1H, m, 4'-H), 3.642 (1H, m, 5'-βH), 3.385 (1H, d, J=12.9Hz, 2'-βH), 3.120 (1H, d, J=10.6Hz, 6'-βH), 2.437 (1H, d, J=12.9Hz, 2'-αH), 2.238 (3H, s, N-$CH_3$), 2.230 (3H, s, 2-$CH_3$), 2.097 (1H, m, 6'-αH) and 1.961 (1H, d, J=10.7Hz, 6'-αH). $^{13}C$-Nmr ($CDl_3$): 182.49 (C4), 165.87 (C7), 165.53 (2C, C=O), 163.10 (C10), 160.95 (C5), 156.49 (C2), 131.51 (4C), 131.20 (2C), 131.10 (4C), 128.45, 128.38, 108.46 (C6), 104.90 (C9), 103.78 (C8), 100.23 (C3), 70.33 (C3'), 56.39 (C2'), 54.19 (C6'), 44.45 (N-$CH_3$), 34.26 (C4'), 23.99 (C5') and 20.27 ($CH_3$).

Fraction 8-13 of the LH-20 chromatography upon evaporation gave a residue containing only one major compound, which was purified by chromatography on a silica gel 60 (230 - 400 mesh, E. Merck) column. Elution of the column with a linear gradient of MeOH in $CHCl_3$ (0 - 5%) gave 26 mgs of colorless needles, mp 177-178°, $^{1}H$-nmr ($CHCl_3$), δ 6.76 (IH,bs), 6.16 (1H,s), 5.36 (1H, dd, J=10.3, 4.2Hz), 3.65 (1H, d, J=3.5Hz), 3.45 (1H, dd, J=10.3,4.2Hz), 2.43 (1H,bs), 2.10 (6H, 2 x OAc), 2.04 (1H, m), 0.89 (3H, s), 0.83 (6H,s), 0.82 (3H,s), 0.79(3H,s), $^{13}C$ nmr $CDCl_3$). 171.35, 170.06, 135.34, 114.44, 101.66, 80.49, 69.32, 64.19, 58.74, 56.49, 54.65, 42.70, 42.09, 41.81, 38.89, 38.05, 37.40, 33.22, 29.67, 27.99, 27.19, 21.36, 21.22, 21.05, 18.56, 18.16, 17.31, 16.30

8.74. Ms 488 ($M^+$). With the exception of C4 position in the $^{13}C$ spectrum (Literature[3] 33.1 this report 29.7 ppm) all carbon values were within 0.5 ppm to that what has been reported for heteronemin.[3]

*Synthesis of I: 2.5 mg of rohitukine[6] was mixed with p-bromobenzoyl chloride and was allowed to* stand for 12 hours, after which the solution was warmed gently for 2 hours. The reaction mixture was allowed to cool and poured into crushed ice. The solution was extracted with chloroform and purified by chromatography on RP-8. The mp and $^1H$-nmr spectrum of the synthetic I was indistinguishable from those of natural I.

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