HETEROCYCLES, Vol. 23, No. 11, 1985

AMATHAMIDE ALKALOIDS FROM THE MARINE BRYOZOAN AMATHIA WILSONI KIRKPATRICK

Adrian J. Blackman* and David J. Matthews
Chemistry Department, University of Tasmania,
G.P.O. Box 252C, Hobart, Tasmania, Australia, 7001

Abstract — Two brominated proline-derived alkaloids named amathamide A and B have been isolated from the marine bryozoan *Amathia wilsoni.*

Bryozoa (Phylum Ectocarpa) are colonial invertebrates, usually of marine origin, whose chemistry has been relatively little investigated. A variety of nitrogenous metabolites containing the physotigmine, indole, quinoline, bipyrrole and purine ring systems have been recently reported.[1]

Coastal waters of southern Australia contain a diverse bryozoan fauna and although colonies are generally small and inconspicuous several have erect, bushy forms which may exceed 100 cm in height.[2] *Amathia wilsoni* Kirkpatrick, 1888, is a reasonably common bryozoan which is found in the eastern coastal regions of Tasmania. Its foliose colonies are typically 40 cm high. The chemical constituents of this animal were investigated as part of our survey of Tasmania's marine natural products; we were led to this organism since it gave a positive Mayer's test for alkaloids.

The alkaloid-containing fraction from a methanol extract of *Amathia wilsoni* was chromagraphed on silica gel to yield two major alkaloids which we have named amathamide A and B, 1 and 2, as well as minor quantities of other bases which are still being investigated.

Amathide A, 1, was obtained as a colourless solid, mp 189-190.5°C in 0.05% yield. High resolution mass spectroscopy indicated the molecular formula $C_{15}H_{18}Br_2N_2O_2$. Initial analysis of the ^1H and ^{13}C nmr data revealed the presence of a tetrasubstituted benzene ring, a disubstituted ethene, and an N-monosubstituted amide as well as an aromatic methoxy and an N-methyl group. The two aromatic protons both occurred as singlets (δ 7.67 and 7.00) in the high resolution ^1H nmr spectrum suggesting that they were *para* to each other. A nuclear Overhauser effect (n.O.e.) was observed from one of the ethene protons (C-8-H) and from the methoxy group when the aromatic proton at higher field (C-15-H) was irradiated. This showed that a styryl moiety was present which had a methoxy group in the *meta* position; the remaining two substituents in the aromatic ring were bromine atoms. Placement of the two bromine atoms in the benzene ring was supported by the observed low intensity of two of the quaternary signals in the ^{13}C nmr spectrum. Aromatic carbons bearing bromine atoms are known to give signals of reduced intensity as a result of carbon-bromine scalar interactions.[3] Confirmation of the presence of the 2,4-dibromo-5-methoxystyryl group was obtained by ozonolysis. Amathamide A gave 2,4-dibromo-5-methoxybenzaldehyde, 3, which was reduced and debrominated with lithium aluminium hydride to *m*-methoxybenzyl alcohol.

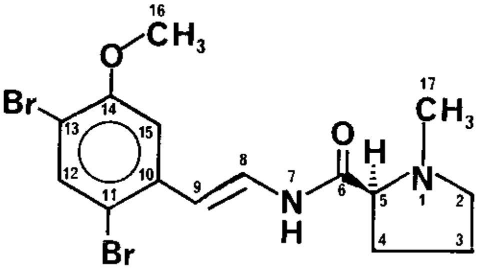
The ethenyl protons in the ^{1}H nmr spectrum occurred as a doublet (δ 6.43, J 14.6 Hz, C-9-H) and a doublet of doublets (δ 7.47, J 14.6, 11.6 Hz, C-8-H) which indicated a *trans* arrangement of the double bond which was in agreement with the n.O.e. data. Since the signal from the low field proton (C-8-H) collapsed to a doublet on adding D_2O the further coupling must have been to the amido N-H (N-7-H). Amathamide A is therefore an *N*-styrylamide. The remaining part of the molecule was assigned to a 2-(1-methylpyrrolidinyl) group. The base peak in the mass spectrum occurred at m/z = 84, a characteristic fragment ion formed from 1-methylpyrrolidines.[4] ^{1}H and ^{13}C spectral data,

Table of ^{13}C chemical shifts (ppm)

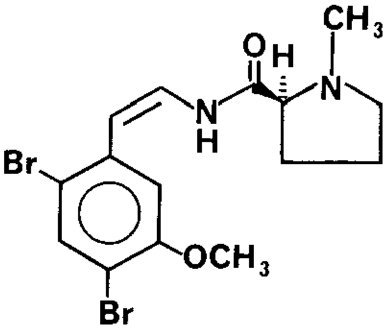
Carbon	Amathamide A	Amathamide B
2	68.73	68.97
3	31.11	31.00
4	24.51	24.47
5	56.70	56.74
6	172.65	172.38
8	124.61	123.00
9	108.29	108.76
10	136.16	135.80
11	113.67*	114.61*
12	136.32	136.89
13	110.40*	110.83*
14	155.44	155.46
15	111.34	112.00
16	56.53	56.35
17	41.98	42.15

Solvent $CDCl_3$

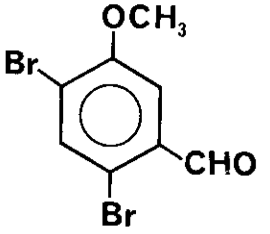
*assignments may be reversed



1



2



3

see Table, fully supported the presence of this group and showed that the point of attachment must be at the 2-position in the pyrrolidine ring. The nmr data for this part of Amathamide A are in close agreement with that of similar compounds, for example nicotine.[5]

Amathamide A thus contains a moiety which is proline-derived. This part of the molecule also contains the only chiral centre and so the absolute configuration was determined by correlation with proline. The circular dichroism spectrum of amathamide A showed a molecular ellipticity of 4000 at 212 nm which is typical of (S)-proline and its simple derivatives.[6] Amathamide A is therefore 2(S)-N-[(E)-2-(2,4-dibromo-5-methoxyphenyl)ethenyl]-1-methyl-2-pyrrolinecarboxamide.

The second major alkaloid isolated from Amathia wilsoni was amathamide B, 2, a colourless oil (0.06% yield) having a somewhat higher R_f than amathamide A. The spectral data of amathamide B were very similar to those of amathamide A. The only significant spectral difference was the magnitude of the coupling constant between the ethenyl hydrogens in the ^{1}H nmr spectrum. In amathamide B the coupling was 9.7 Hz compared to 14.6 Hz in amathamide A showing that amathamide B has a cis arrangement about the double bond and is therefore 2.

The amathamides are presumably biosynthesised from proline and phenylalanine. The methoxy group is unusually positioned being in the meta rather than the more common para position of the aromatic ring and suggests that tyrosine is not a biosynthetic precursor.

EXPERIMENTAL

Extraction and isolation procedure

Specimens of Amathia wilsoni were collected at Oyster Bay, Maria Island in November 1981. A voucher specimen has been deposited in the Invertebrate Zoology Collection of the National Museum of Victoria, registry no. F51055. The bryozoan was stored at -10°C prior to extraction. After soaking the bryozoan (1.75 kg extracted dry weight) overnight in methanol, the extract was decanted and the residue further extracted until a negative Mayer's test was obtained. The combined methanol extract was reduced in vacuo at 32°C to 3l. This was acidified by adding 200 ml 2M H_2SO_4 and washed with petroleum bp 40-60°C to remove lipids. The methanol extract was then treated with 100 ml of conc ammonium hydroxide and extracted with dichloromethane until the extracts gave a negative result with Mayer's reagent. After drying over anhyd Na_2SO_4, evaporation of the dichloromethane solution gave a brown residue which, after application to a dry column of silica gel and gradient elution with dichloromethane containing increasing quantities of methanol, afforded alkaloidal material (2.38 g). A portion (250 mg) of this extract gave amathamide A as a white crystalline solid, mp 189-190.5°C (90 mg, R_f 0.5, 0.05% yield on dry wt.) and amathamide B, a colourless oil (110 mg, R_f 0.6, 0.06% yield on dry wt.) by PTLC on Camag DFS-5 silica gel using multiple development with dichloromethane containing 2% methanol.

Ozonolysis of Amathamide A

Amathamide A (650 mg, 1.51 mmole) was dissolved in dichloromethane (30 ml), cooled to -56°C in an acetone-dry ice bath and saturated with ozone. Triphenylphosphine (600 mg, 2.23 mmole) was then added and the solution allowed to come to room temperature. Evaporation of the solvent followed by silica gel chromatography gave the aldehyde (440 mg, 1.51 mmole) 3, mp 95°C; ^{1}H nmr (100 MHz, $CDCl_3$); δ 10.10 (1H, s), 7.73 (1H, s), 7.30 (1H, s), 3.90 (3H, s); ms, m/z: 292 (M^+) (Br_2), 291 (Br_2), 277 (Br_2), 221 (Br_2), 197 (Br), 184 (Br), 170 (Br), 156 (Br), 141 (Br).

Treatment of 3 (120 mg, 0.41 mmole) with lithium aluminium hydride (93 mg, 2.45 mmole) in refluxing dioxan for 2 h gave m-methoxybenzyl alcohol (28 mg, 0.20 mmole) as a colourless oil which was identical with authentic m-methoxybenzyl alcohol prepared from m-hydroxybenzaldehyde.

Spectral Analysis

Nmr spectra of amathamides A and B were recorded on a Bruker CXP300 spectrometer at the Griffith University NMR Laboratory, Qld. N.O.e. data were obtained on a Bruker CXP300 spectrometer at the University of NSW while ^{1}H nmr spectra of the ozonolysis products were determined on a Jeol JNM-4H-100 instrument. Ir spectra were obtained on a Digilab FTS-IMX spectrometer and mass spectra on a Vacuum General Micromass 7070F spectrometer.

Amathamide A: hrms $C_{15}H_{18}{}^{79}Br^{81}BrN_2O_2$, meas. 417.9694, calc. 417.9714; ms, m/z: 416 (M^+, Br_2), 210 (Br), 182 (Br), 84; ^{1}H nmr (300 MHz, $CDCl_3$): δ 9.32 (d, J 11.55 Hz, 1H, NH), 7.67 (s, 1H, ArH), 7.47 (dd, J 14.55, 11.55 Hz, 1H, =CH), 7.00 (s, 1H, ArH), 6.43 (d, J 14.57 Hz, 1H, =CH), 3.91 (s, 3H, OCH_3), 3.19 (m, 1H), 3.03 (dd, J 10.37, 4.96 Hz, 1H), 2.44 (s, 3H, NCH_3), 2.44 (m, 1H), 2.29 (m, 1H), 1.92 (m, 1H), 1.86 (m, 2H); ir ν_{max} (CH_2Cl_2) cm^{-1}: 3259, 1676, 1643, 1497, 1437, 1371; CD, (95% ethanol, 3.78 mM, 21°C):$[\theta]_{212}$ 4000.

Amathamide B: m/z 416 (M^+, Br_2), 210 (Br), 182 (Br), 84; ^{1}H nmr (300 MHz, $CDCl_3$): 9.45 (d, J 12.12 Hz, 1H, NH), 7.78 (s, 1H, ArH), 7.04 (dd, J 12.12, 9.70 Hz, 1H, =CH), 6.90 (s, 1H, ArH), 5.75 (d, J 9.73 Hz, 1H, =CH), 3.89 (s, 3H, OCH_3), 3.03 (m, 2H), 2.36 (s, 3H, NCH_3), 2.33 (m, 2H), 1.80 (m, 3H); ir ν_{max} (CH_2Cl_2) cm^{-1}: 3310, 1689, 1647, 1489, 1461.

ACKNOWLEDGEMENTS

We thank Dr Phil Bock, National Museum of Victoria, for identifying the bryozoan and Mr Brian Fleming for assistance with its collection. We are obliged to Ms V. M. J. Robson, Monash University for measurement of the circular dichroism spectrum, Dr John Saunders for obtaining the n.O.e. data and Dr Peter Barron for nmr spectra. Financial support by the Australian Research Grants Scheme is gratefully acknowledged.

REFERENCES

1. D. J. Faulkner, Natural Products Reports, 1984, 1, 551.
2. P.E. Bock, 'Marine Invertebrates of Southern Australia', ed. by S. A. Shepherd and I. M. Thomas, Government Printer, 1982, p319.
3. R. S. Norton, Tetrahedron, 1977, 33, 2577.
4. E. Pretsch, T. Clerc, J. Seibel and W. Simon, 'Tables of Spectral Data for Structure Determination of Organic Compounds', Springer-Verlag, 1983.
5. E. Wenkert, J. S. Bindra, C.-J. Chang, D. W. Cochran and F. M. Schell, Accounts Chem. Res., 1974, 7, 46.
6. J. Cymerman Craig and W. E. Pereira, Tetrahedron, 1970, 26, 3457.

Received, 24th June, 1985