Pergamon

Tetrahedron Letters, Vol. 38, No. 6, pp. 941-944, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(97)00003-8

Aplidiamine, a Unique Zwitterionic Benzyl Hydroxyadenine from the Western Australian Marine Ascidian *Aplidiopsis* sp.

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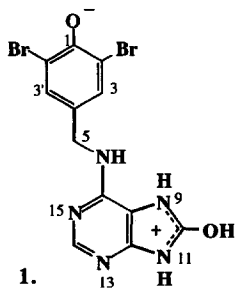
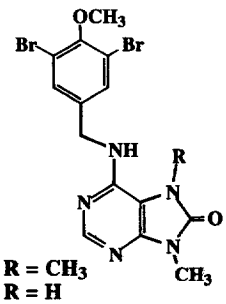
Abstract: Aplidiamine (**1**), a unique zwitterionic benzyl substituted hydroxyadenine, has been isolated from the marine ascidian *Aplidiopsis* sp. collected in Western Australia. The structure of aplidiamine was determined by spectral analysis of the natural product and of several derivatives produced by diazomethane methylation. © 1997, Elsevier Science Ltd. All rights reserved.

Ascidians are rich sources of bioactive secondary metabolites derived mainly from amino acids.[1] As part of a comprehensive chemical investigation of the ascidians from the Ningaloo Reef region in Western Australia, we evaluated the secondary metabolite chemistry of an undescribed *Aplidiopsis* sp. (Polycitoridae, Ascidaeae) and wish to describe here the structure of aplidiamine (**1**),[2] a unique zwitterionic benzyl adenine of an unprecedented structural class.

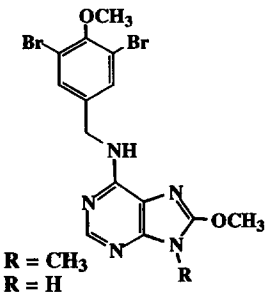
The frozen animal (3.5 kg, wet wt) was lyophilized, the dry tissue macerated, and extracted three times with 70% MeOH/CH_2Cl_2. The combined extract was concentrated under vacuum and partitioned into hexane, ethyl acetate, *n*-butanol and water. Repetitive gel filtration of the ethyl acetate fraction by Sephadex LH-20 column chromatography (MeOH) gave pure aplidiamine (**1**, 20 mg, R_f = 0.1 with 10 % MeOH/DCM on silica TLC) as a white solid, mp 241-243 °C, after precipitation from MeOH. Aplidiamine (**1**) analyzed for $C_{12}H_9N_5O_2Br_2$ by HRFABMS and combined spectral methods, a formula which indicated 10 degrees of unsaturation. Since only six proton resonances were observed in the ^1H NMR spectrum of **1**, the molecule was recognized to possess an element of symmetry. A bathochromic shift upon addition of base, in conjunction with a strong IR absorption at 3300 cm^{-1}, suggested aplidiamine contained an acidic hydroxyl functionality.



1.



2. R = CH_3
3. R = H



4. R = CH_3
5. R = H

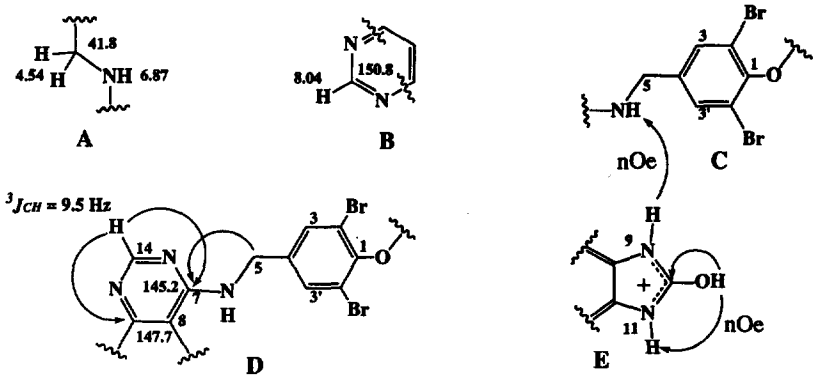
Table. NMR Data for Aplidiamine (1) in DMSO-d_6

C &N #.	^{13}C	^{1}H	HMBC (8 Hz)	NOESY
C-1	149.9			
C-2, 2'	111.9			
C-3, 3'	131.2	7.52 (s, 2H)	C1, C2, C2', C3', C3, C5	H5
C-4	134.0			
C-5	41.8	4.54 (d, 2H, J = 5.5 Hz)	C3, C3', C4, C7	H3, H3', H6,
N-6		6.87 (t, 1H, J = 5.5 Hz)	C5, C7, C8	H5, H9
C-7	145.2			
C-8	104.7			
N-9		9.87 (s, 1H)	C8, C10	H6
C-10	152.7	9.83 (s, OH)	C10	H11
N-11		11.33 (s, 1H)	C8, C10, C12	H10
C-12	147.7			
C-13				
C-14	150.8	8.04 (s, 1H)	C7, C12	

All spectra were recorded in DMSO-d_6 at 500 MHz (^{1}H) and 125 MHz (^{13}C). Chemical shifts are reported δ units (downfield of TMS). Assignments were based upon HMQC experiments.

The proton spectrum of **1** indicated the presence of an *N*-alkylated primary amine (substructure **A** below) [δ 4.54 (d, 2H, J = 5.5 Hz, CH_2) and 6.87 (t, 1H, J = 5.5 Hz, NH)]. In addition, the spectrum showed two aromatic singlets (δ 7.52 and 8.04), and three exchangeable protons [δ 9.83, 9.87 and 11.3]. The proton signal at δ 8.04 was shown by HMQC analysis to be directly coupled to a carbon signal at δ 150.8. The measured $^{1}J_{CH}$ coupling constant between this proton and the carbon was 200 Hz, a large value which can be accommodated by several structures: furan ($^{1}J_{C\text{-}2H\text{-}2}$ = 202 Hz), imidazole ($^{1}J_{C\text{-}2H\text{-}2}$ = 208 Hz), triazole ($^{1}J_{C\text{-}2H\text{-}2}$ = 209 Hz) and pyrimidine ($^{1}J_{C\text{-}2H\text{-}2}$ = 203 Hz).[3] Pyrimidine substructure **B** was the most consistent based upon the molecular formula for **1** and on the observed proton and carbon chemical shifts of C7, C8, C12 and C14. Combined HMQC and HMBC spectral data also established the presence of a 1, 2, 3, 5-tetrasubstituted benzene ring. A proton signal at δ 7.52 (H3/H3') correlated to a carbon signal at δ 131.2 (C3/C3') in the HMQC spectrum. The same proton also showed long range HMBC correlations to two quaternary carbon signals at δ 149.9 (C1) and 111.9 (C2/C2'), thus defining a symmetrical benzene ring. By virtue of their chemical shifts, the two carbons at δ 111. 9 were assigned as the bromine bearing carbons while the deshielded carbon (δ 149.9) was assigned as oxygen bearing. The benzene ring was next connected to substructure **A** based on HMBC correlations of the C5 methylene protons signal (δ 4.54) with the two benzene carbon signals at δ 131.2 (C3/C3') and 134.0 (C4). Linking these part structures resulted in establishing partial structure **C**. The dibromophenolic moiety is a well known component in many marine metabolites, examples being the sponge metabolites of the bastidin and the purealidin classes.[4] The spectral data for this component of aplidiamine were fully consistent with other analogous bromophenols.

Next, the benzyl amine moiety was connected to the purine ring at C7 on the basis of HMBC correlations. A quaternary carbon at δ 145.2 (C7) showed long range correlations to both proton signals at δ 4.54 (H5) and 6.87 (H6), while a quaternary carbon at δ 104.7 (C8) only correlated to the proton signal at δ 6.87. Furthermore, the former carbon signal was coupled to an aromatic singlet at δ 8.04 (H14). Since $^{4J}_{CH}$ coupling was not likely in the HMBC (8 Hz) spectrum, the carbon signal at δ 145.2 was assigned to C7. The proton signal at δ 8.04 showed additional coupling to a quaternary carbon at δ 147.7, assigned as C12. On the basis of these data, a 2-amino-3, 4-disubstituted pyrimidine structure (**D**) was identified. In the HMBC



spectrum of **1**, both $^3J_{H4\text{-}C7}$ and $^3J_{H14\text{-}C12}$ coupling constants were accurately measured in the proton domain since the carbon domain was not decoupled. Both coupling constants were 9.5 Hz, values consistent with literature models.[3] The remaining carbon signal at δ 152.7 (C10), in conjunction with a strong IR absorption at 1705 cm^{-1}, suggested the presence of a cyclic urea component, thus suggesting **1** to be a benzyl substituted adenine-2-one. However, this was not consistent with HMBC results observed with aplidiamine in solution. Three exchangeable protons at δ 9.83 (OH), 9.87 (NH) and 11.3 (NH), which showed HMBC correlations with three carbon signals at δ 104.7, 147.7, and 152.7, suggested the remaining 5-membered ring in aplidiamine to be composed of a 2-hydroxy-imidazolium functionality. To further probe the composition of this unique compound, aplidiamine was methylated with excess diazomethane in $MeOH/CH_2Cl_2$. The reaction yielded four methylated products, **2-5**[5], which were fully characterized. Methyl derivatives **2** and **4** provided additional evidence for structure **1** since the new *N*-methyl signals provided additional long range NMR heterocorrelations.

Compound **2**[5] analyzed for $C_{15}H_{15}N_5O_2Br_2$, by HREIMS (M^+ obsd. *m/z* 458.9547 dev. -1.0 ppm), confirming that **2** was a trimethyl derivative of **1**. HMBC analysis showed that an *N*-methyl signal (δ 3.65) at N9 coupled to C8 (δ 105.6) and C10 (δ 153.0). An *N*-methyl signal (δ 3.45) at N11 also showed two correlations to C10 (δ 153.0) and C12 (δ 147.4) in the HMBC spectrum of **2**, thus illustrating the presence of an imidazol-2-one moiety.

Compound **4**[5] analyzed for $C_{15}H_{15}N_5O_2Br_2$ by HREIMS (M^+ obsd. *m/z* 458.9568 dev. 3.6 ppm), a formula which illustrated that **4** was a methyl regioisomer of **2**. The proton NMR spectrum of compound **4** was almost identical to that of **2**, except for the presence of an additional methoxy signal at δ 4.15 (s, 3H) replacing an *N*-methyl at δ 3.65 (s, 3H) in **2**. The methoxy protons showed an HMBC correlation to C10 (δ 155.4), leading to assignment of compound **4** as the corresponding imine. Two additional products, **3** and **5**, were characterized based on a comparison of ^1H NMR assignment for **2** and **4**. All spectral data were consistent with the proposed structures **3** and **5**.[5]

The assignment of aplidiamine (**1**) as a zwitterionic structure in DMSO-d_6 solution is based upon the presence of a hydroxyl proton at C10, which is observed in the HMBC spectrum to be coupled to C10 (δ 152.7) and not to C1 (δ 149.9). Furthermore, methylation of aplidiamine yielded four products which were consistent with this proposition. Additional support for this assignment was obtained from the results of NOESY and HMBC experiments (Table) which clearly showed that all three exchangable protons were adjacent to one another. The NOESY experiment also confirmed the spatial proximities of the benzyl protons and the benzyl amine proton. Based upon IR data, however, aplidiamine appears to exist as a mixture of neutral and

zwitterionic forms in the solid state.[6] Absorptions at 1701, 2700-2400 (m) and 1682 cm^{-1}, which are C=O absorptions (from adenin-2-one), and an ammonium band (N^+-H) and immonium ($C=N^+$) band (from a positively charged adenin-2-ol) respectively, fully support this conclusion.

The mass spectral features of **1** supported the proposed structure, although data could not differentiate between a zwitterionic or neutral form. A fragment ion consistent with a *p*-hydroxy dibromobenzylic amine subunit (cleavage at the C7-N6 bond) was observed at *m/z* = 277, 279, and 281, illustrating its bromine isotopic composition. The presence of the adenine-8-ol moiety was supported by a strong fragment ion at *m/z* = 152.

Aplidiamine appears to have an unusual biosynthetic origin. Most compounds possessing the dibromophenol subunit[4] are thought to be derived from tyrosine. However, aplidiamine possesses a benzyl amine rather than a phenethyl amine, as would be observed in a tyrosine-derived metabolite. Curiously, the structure of aplidiamine has similar features to some cytokinins.[7] Aplidiamine is an interesting example of an ascidian metabolite which could easily be neglected by virtue of the simplicity of its spectral data and high polarity.

Acknowledgements

This research is a result of financial support from the National Science Foundation, Chemistry Division, under grant CHE93-22776, and in part from the California Sea Grant College Program (grant # NA89AA-D-SD138, project RM/P-59). H. K. would like to acknowledge the Korean Government Oversea Fellowship and the California State Sea Grant Program for support in the form of a Sea Grant Traineeship. We thank Dr. François Monniot for identifying this ascidian.

References and Notes

1. Davidson, B. S. *Chem. Rev.* **1993**, *93*, 1771.

2. Additional data for aplidiamine (**1**): white, water-soluble solid, mp 241-243 °C; HRFABMS: $(M + H)^+$ obsd. *m/z* 413.9189, $C_{12}H_{10}N_5O_2Br_2$, dev. -3.0 ppm; IR (NaCl) ν_{max}: 3700-3000, 1701, 1682, 1634 cm^{-1}; UV (50 % $MeOH/CH_2Cl_2$) λ_{max}: 273 (ε 15800), 221 (17300) nm; UV ($MeOH/CH_2Cl_2$ + HCl) λ_{max}: 290 (ε 15200), 223 (19000) nm; UV ($MeOH/CH_2Cl_2$ + NaOH) λ_{max}: 377 (sh), 313 (sh), 283 (ε 30700), 250 (sh), 223 (52500) nm.

3. Breitmaier, E.; Voelter, W. *Carbon-13 NMR Spectroscopy: High-Resolution Methods and Applications in Organic Chemistry and Biochemistry*; VCH publishers: New York, 1987; pp. 515.

4. a) Pordesimo, E. O.; Schmitz, F. J. *J. Org. Chem.* **1990**, *55*, 4704. b) Tsuda, M.; Shigemori, H.; Ishibashi, M.; Kobayashi, J. *Tetrahedron Lett.* **1992**, *33*, 2597.

5. For derivative **2**: amorphous solid, HREIMS: M^+ obsd. *m/z* 458.9547, $C_{15}H_{15}N_5O_2{}^{81}Br_2$, dev. -1.0 ppm; IR (NaCl) ν_{max}: 3360, 1702, 1618 cm^{-1}; UV (diode-array HPLC) λ_{max}: 275, 220 nm; ^1H NMR (500MHz, $CDCl_3$): δ 3.45(s, 3H), 3.65 (s, 3H), 3.88 (s, 3H), 4.71 (d, 1H, *J* = 6Hz), 4.99 (t, 1H, *J* = 6Hz), 7.51 (s, 2H), 8.27 (s, 1H). For derivative **3**: amorphous solid, HREIMS: M^+ obsd. *m/z* 440.9467, $C_{14}H_{13}N_5O_2{}^{79}Br_2$, dev. 7.0 ppm; IR (NaCl) ν_{max}: 3368, 1618, 1567 cm^{-1}; UV (diode-array HPLC) λ_{max}: 275, 220 nm; ^1H NMR (500MHz, $CDCl_3$): δ 3.28 (s, 3H), 3.86 (s, 3H), 4.75 (d, 2H, *J* = 5.8 Hz), 6.73 (bs, 1H), 7.50 (s, 2H), 8.28 (s, 1H). For derivative **4**: amorphous solid, HREIMS: M^+ obsd. *m/z* 458.9568, $C_{15}H_{15}N_5O_2{}^{81}Br_2$, dev. 3.6 ppm; UV (diode-array HPLC) λ_{max}: 275, 220 nm; ^1H NMR (500MHz, $CDCl_3$): δ 3.56(s, 3H), 3.86 (s, 3H), 4.15 (s, 3H), 4.79 (d, 1H, *J* = 6Hz), 5.80 (bs, 1H), 7.53 (s, 2H), 8.31 (s, 1H). For derivative **5**: amorphous solid, HREIMS: M^+ obsd. *m/z* 440.9449, $C_{14}H_{13}N_5O_2{}^{79}Br_2$, dev. 3.0 ppm; UV (diode-array HPLC) λ_{max}: 275, 220 nm; ^1H NMR (500MHz, $CDCl_3$ w/ MeOH-d_4): δ 3.84 (s, 3H), 3.86 (s, 3H), 4.65 (s, 2H), 7.49 (s, 2H), 7.89 (s, 1H).

6. Nakanishi, K. *Infrared Absorption Spectroscopy*, Holden-Day, Inc.: San Francisco, 1962; pp. 233.

7. a) Helgeson, J. P. *Science* **1968**, 161, 974. b) Chen, C.; Smith, O. C.; McChesney, J. D. *Biochemistry* **1975**, 14, 3088.

(*Received in USA* 16 *September* 1996; *revised* 17 *December* 1996; *accepted* 18 *December* 1996)