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ASCIDIDEMIN, A NOVEL PENTACYCLIC AROMATIC ALKALOID WITH POTENT ANTILEUKEMIC ACTIVITY FROM THE OKINAWAN TUNICATE DIDEMNUM SP.

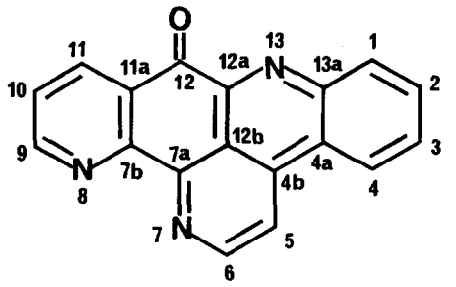
Jun'ichi Kobayashi*, Jie-fei Cheng, Hideshi Nakamura, Yasushi Ohizumi
Mitsubishi-Kasei Institute of Life Sciences, Minamiooya, Machida, Tokyo 194, Japan
Yoshimasa Hirata
Faculty of Pharmacy, Meijo University, Nagoya 468, Japan
Takuma Sasaki
Cancer Research Institute, Kanazawa University, Kanazawa 920, Japan
Tomihisa Ohta and Shigeo Nozoe
Pharmaceutical Institute, Tohoku University, Sendai 980, Japan

Summary: A novel pentacyclic aromatic alkaloid, ascididemin (**1**), with potent antineoplastic activity has been isolated from the Okinawan tunicate Didemnum sp. Its structure was elucidated on the basis of spectroscopic data.

Tunicates have proven to be a good source of pharmacologically active compounds, like didemnins[1] or eudistomins[2] with antitumor or antiviral activities, respectively. In our continuous survey on bioactive metabolites from marine organisms,[3] methanol extracts of a tunicate was found to show potent antileukemic activity. We report here the isolation and the structure elucidation of a novel pentacyclic aromatic alkaloid, named ascididemin (**1**), with powerful antineoplastic activity from the Okinawan tunicate Didemnum sp.

The brown-colored compound tunicate collected at Kerama Islands, Okinawa, was kept frozen until used. The methanol extract was partitioned between ethyl acetate and water. The ethyl acetate-soluble material, exhibiting antileukemia activity, was subjected to silica gel column chromatography ($CHCl_3$/MeOH, 95:5) followed by repeated precipitation with chloroform to afford ascididemin (**1**) (0.006%, wet weight) as a yellow solid: mp > 300°C; IR(KBr) ν_{max} 1680, 1600, 1580, 1410, 1260, 860, and 740 cm^{-1}; UV(MeOH) λ_{max} 220 (ε 49500), 248 (48000), 273 (sh 27500), 298 (17000), 308 (15700), 340 (sh 11300), and 377 (13600) nm.

The molecular formula $C_{18}H_9ON_3$ for **1**, implying 16 degrees of unsaturation, was determined by HRFABMS (m/z 286.1006, M^++2+H, Δ 2.5 mmu). The FABMS pattern showing only a (M^++2+H) peak as a pseudomolecular ion peak is similar to those of quinones[4] or iminoquinone compounds.[5] The EIMS showed a molecular ion peak at m/z 283 and fragment peaks at m/z 255 (M^+-CO), 228 (M^+-CO-HCN) and 200 (M^+-CO-2HCN). The ^{1}H NMR spectrum (Table 1) showed only 9 aromatic protons which were assignable to 4 protons (δ 8.55, dd, J=1.3 and 7.7 Hz, H-1; 8.05, ddd, J=1.3, 7.7 and 8.1 Hz, H-2; 7.99, ddd, J=1.3, 7.7 and 8.1 Hz, H-3; 8.76, dd, J=1.3 and 7.7 Hz, H-4) on a benzene ring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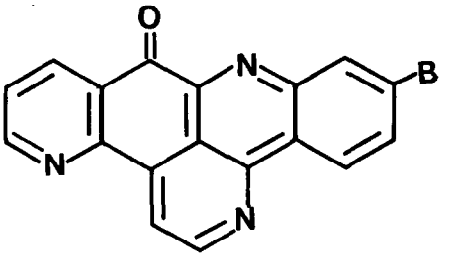
11
11a
12
12a
13
13a
1
10
2
9
12b
3
N
7a
4a
7b
4b
4
8
7
N
5
6

1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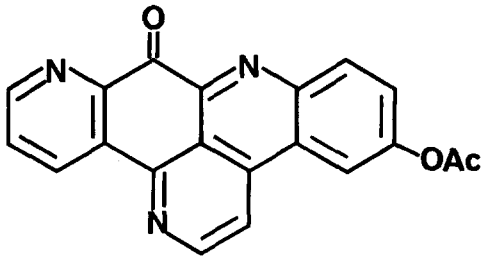
N
B
N
N

2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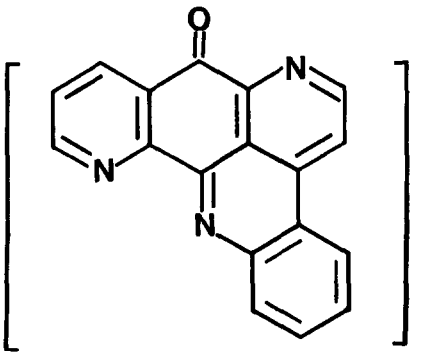
N
OAc
N

3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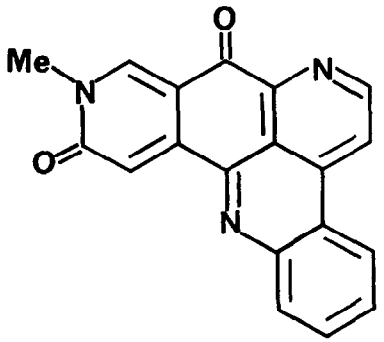
N
N

4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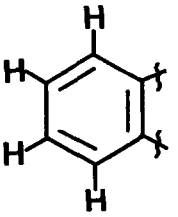
O
N
O
N
N

5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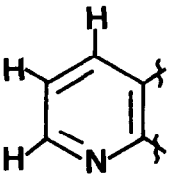
H
H
H

A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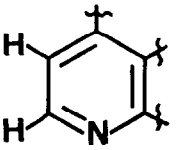
H
H
N

B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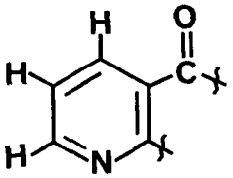
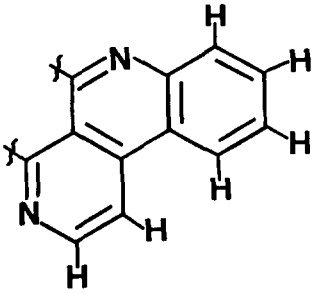
C


H
O
H
C
H
N

D


H
N
H
H
H
N
H
H

E

Table 1. ^{1}H and ^{13}C NMR Data for Ascididemin (1)

C	δC^{a} (m)	δH^{b} at C (m)	J, Hz	Protons coupled with C^{c}
1	132.79 (d)	8.55 (dd)	1.3,7.7	H-3
2	132.52 (d)	8.05 (ddd)	1.3,7.7,8.1	H-4
3	131.53 (d)	7.99 (ddd)	1.3,7.7,8.1	H-1
4	123.59 (d)	8.76 (dd)	1.3,7.7	H-2
4a	123.91 (s)			H-1,H-3,H-5
4b	138.51 (s)			H-4,H-6
5	117.70 (d)	8.69 (d)	5.6	H-6
6	149.87 (d)	9.22 (d)	5.6	H-5
7a	149.67 (s)			H-6
7b	152.38 (s)			H-9,H-11
9	155.67 (d)	9.14 (dd)	1.7,4.7	H-11
10	126.30 (d)	7.75 (dd)	4.7,7.7	H-9
11	136.99 (d)	8.79 (dd)	1.7,7.7	H-9
11a	129.24 (s)			H-10
12	181.99 (s)			H-11[d]
12a	145.94 (s)			
12b	118.24 (s)			H-5
13a	145.75 (s)			H-2,H-4

[a] 125 Hz, $CDCl_3/CD_3OD$ (3.5 : 1.5). [b] 500 MHz, $CDCl_3/CD_3OD$ (3.5 : 1.5). [c] Observed in COLOC experiments (J_{max}=10 Hz). [d] Observed in J_{max}=5 Hz.

(partial structure A), 3 protons (δ 9.14, dd, J=1.7 and 4.7 Hz, H-9; 7.75, dd, J=4.7 and 7.7 Hz, H-10; 8.79, dd, 1.7 and 7.7 Hz, H-11) on a disubstituted pyridine ring (partial structure B), and 2 protons (δ8.69, d, J=5.6 Hz, H-5; 9.22, d, J=5.6 Hz, H-6) on a trisubstituted pyridine ring (partial structure C). In addition to the partial structures A-C the connectivity of C-4a to C-4b was confirmed by the COLOC experiments,[6] in which the long-range couplings were observed between H-4 and C-4b, and H-5 and C-4a (Table 1). This connectivity was also supported by a NOE enhancement (12%) of H-4 on irradiation of H-5. A carbonyl group (δ181.99, IR 1680 cm^{-1}) was attached to C-11a (partial structure D), since a cross peak of H-11 to the carbonyl carbon (C-12) was revealed in the COLOC spectra (J_{max}=5 Hz) and a clear 3-bond coupling (J=3 Hz) has been observed between H-11 and C-12 of 2-bromoleptoclinidinone (**2**)[7] possessing the same partial structure as D. From the comparison of the carbon chemical shifts of C-12b (δ118.24) and C-13a (δ145.75) with the corresponding resonances of **2**[7] (δ117.9 and 146.3) and neocalliactine[8] (**3**, δ117.8 and 143.2), the remaining imino group (δ145.94) was inserted between C-12b and C-13a (partial structure E). Combination of partial structures D and E, however, allowed the two possible structure (**1**) or (**4**). The resonances at C-7a (δ149.67) and C-12a (δ145.94) of **1** were compared with the corresponding ones of **3** and amphimedine[9] (**5**),

possessing the same partial structure of **1** or **4**, respectively. The resonances of **1** were almost equal to those of **3** (δ149.4 and 145.3) but not to **5** (δ145.1 and 139.8). Furthermore, C-7a was coupled with only H-6 in the COLOC experiments (Table 1). The structure of ascididemin was thus concluded to be **1**.

Although the structure of ascididemin (**1**)[10] is closely related to **2** or **5** isolated from a tunicate[7] or a marine sponge,[8] or to **3**, a hydrolysis product of a sea anemone pigment,[9] biosynthetic pathways of these compounds remain to be investigated. Ascididemin (**1**) was cytotoxic with IC_{50} value of 0.39 μg/ml against L1210 murine leukemia cells in vitro, and also seven times more potent than caffeine, a well-known Ca-releaser,[11] in the Ca-releasing activity in sarcoplasmic reticulum.

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10. Although ascididemin (**1**) revealed the presence of nitrogen atoms in the same relative position as in 1,10-phenanthroline, a well-known metal chelating agent, formation of a red complex with iron (II) salts was observed for 1,10-phenanthroline but not for **1**.
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