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THREE NEW CYTOTOXIC ALKALOIDS, APLAMINONE, NEOAPLAMINONE AND NEOAPLAMINONE SULFATE FROM THE MARINE MOLLUSC *APLYSIA KURODAI*

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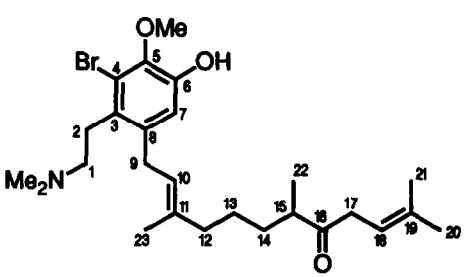
Abstract: Three novel alkaloids, aplaminone (**1**), neoaplaminone (**2**), and neoaplaminone sulfate (**3**) possessing cytotoxic activity have been isolated from the marine mollusc *Aplysia kurodai*. Their structures were elucidated on the basis of spectral and chemical means.

A variety of structurally and pharmacologically interesting compounds have been isolated from the marine mollusc *Aplysia kurodai*.[1] In the course of our investigation on bioactive compounds from *A. kurodai* collected at Azurihama of the Shima Peninsula, Mie Prefecture, Japan, we have isolated three new cytotoxic alkaloids, aplaminone (**1**), neoaplaminone (**2**), and neoaplaminone sulfate (**3**). We describe herein the structural determination of these alkaloids on the basis of the spectral and chemical evidence.

The EtOAc-soluble material from the MeOH extract of *A. kurodai* was partitioned between CCl_4-CH_2Cl_2 (1:1) and H_2O-MeOH (3:7), and subsequently the CCl_4-CH_2Cl_2 (1:1) layer was further partitioned between CCl_4 and MeOH-H_2O (8:2). The MeOH-H_2O (8:2) layer was subjected to chromatography on silica gel (EtOAc → EtOAc-MeOH) and subsequently on alumina [EtOAc → EtOAc-MeOH (9:1 → 1:1)]. The fractions eluted with EtOAc and EtOAc-MeOH (9:1) was separated by chromatography on silica gel [hexane-EtOAc-MeOH (3:3:1)] to give aplaminone (**1**)[2] (colorless oil; 1.6 x 10^{-4}% wet weight) and neoaplaminone (**2**)[3] (colorless oil; 2.4 x 10^{-5}% wet weight). The fractions eluted with EtOAc-MeOH (1:1) was separated by reversed-phase HPLC [ODS, MeOH-H_2O (50:50)] to afford neoaplaminone sulfate (**3**)[4] (colorless oil; 8.3 x 10^{-5}% wet weight).



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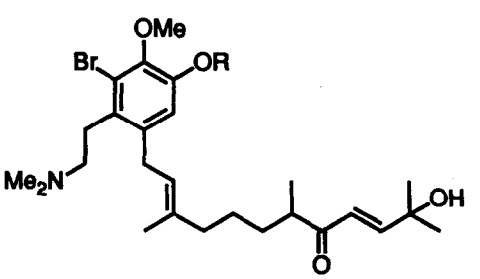


2 R = H
3 R = SO_3H

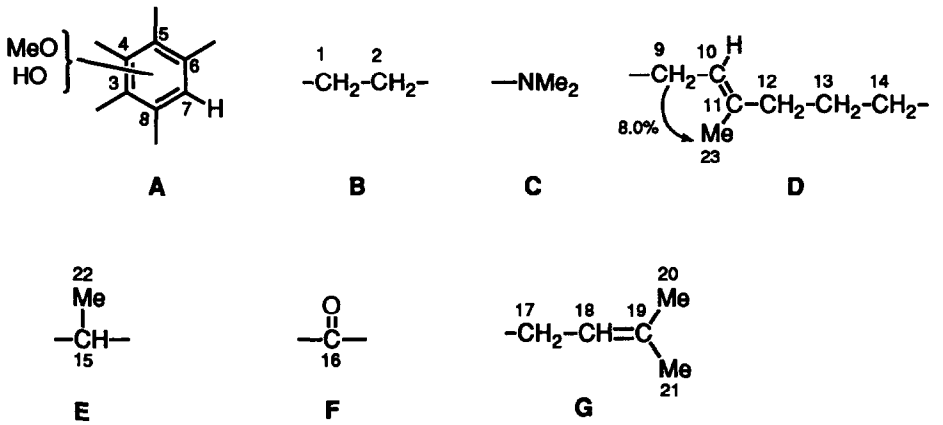
The molecular formula of **1** was determined to be $C_{26}H_{40}BrNO_3$ by high resolution EIMS. The IR spectrum of **1** indicated the presence of a saturated ketone group (1705 cm^{-1}). The spectral data [IR ($CHCl_3$) 3380, 1595 cm^{-1}; UV (MeOH) λ_{max} 285 nm (ε 2,900); ^{13}C NMR (δ_C 129.1 (s), 121.1 (s), 144.3 (s), 150.0 (s), 117.6 (d), 137.8 (s): cf. Table 1); ^1H NMR (δ_H 6.75 (1H, s, aromatic H), 3.77 (3H, s, aromatic OMe): cf. Table 1)] coupled with the positive reaction to the $FeCl_3$-$K_3Fe(CN)_6$ test[5] suggested the presence of the partial structure **A** in **1**. The ^1H and ^{13}C NMR spectra of **1** (Table 1) also revealed the presence of 3 x vinyl Me, 1 x Me_2N-, 1 x secondary Me, 1 x ketonic carbonyl, 2 x -CH=C<, 7 x CH_2, and 1 x -CH-.
These groups were correlated by the detailed analysis of the ^1H-^1H COSY spectrum of **1** to give the partial structures, **B**, **C**, **D**, **E**, **F**, and **G**. The stereochemistry of the double bond in **D** was determined by the NOE

Table 1. ^{13}C and ^1H NMR Spectral Data for Aplaminone (**1**), Neoaplaminone (**2**), and Neoaplaminone Sulfate (**3**) (Acetone-d_6)[a]

Carbon	1		2		3	
No.	δ_C (m)	δ_H(m, *J* in Hz)	δ_C (m)	δ_H(m, *J* in Hz)	δ_C (m)	δ_H(m, *J* in Hz)
1	59.2 (t)	2.37 (m)	59.3 (t)	2.38 (m)	36.8 (t)	2.87 (m)
NMe_2	45.3 (q)	2.28 (s)	45.4 (q)	2.28 (s)	43.9 (q)	2.87 (s)
2	30.5 (t)	2.92 (m)	30.9 (t)	2.92 (m)	28.7 (t)	3.06 (m)
3	129.1 (s)		129.7 (s)		131.1 (s)	
4	121.1 (s)		121.1 (s)		121.0 (s)	
5	144.3 (s)		144.2 (s)		148.2 (s)	
OMe	60.3 (q)	3.77 (s)	60.5 (q)	3.77 (s)	61.2 (q)	3.88 (s)
6	150.0 (s)		149.8 (s)		146.7 (s)	
7	117.6 (d)	6.75 (s)	117.6 (d)	6.76 (s)	124.1 (d)	7.51 (s)
8	137.8 (s)		138.2 (s)		137.7 (s)	
9	32.6 (t)	3.34 (d, 6.9)	32.7 (t)	3.35 (d, 6.9)	32.6 (t)	3.29 (d, 6.6)
10	124.0 (d)	5.22 (m)	124.1 (d)	5.22 (m)	123.4 (d)	5.20 (m)
11	136.7 (s)		136.9 (s)		137.4 (s)	
12	40.2 (t)	2.03 (m)	40.2 (t)	2.05 (m)	40.2 (t)	2.04 (m)
13	26.1 (t)	1.37 (m)	26.1 (t)	1.38 (m)	26.1 (t)	1.38 (m)
		1.43 (m)		1.43 (m)		1.45 (m)
14	33.2 (t)	1.30 (m)	33.5 (t)	1.32 (m)	33.5 (t)	1.32 (m)
		1.67 (m)		1.70 (m)		1.64 (m)
15	45.9 (d)	2.63 (m)	44.4 (d)	2.84 (m)	44.3 (d)	2.87 (m)
16	211.9 (s)		203.9 (s)		204.2 (s)	
17	41.4 (t)	3.17 (d, 6.9)[b]	125.2 (d)	6.38 (d, 15.8)	125.3 (d)	6.38 (d, 15.8)
18	117.5 (d)	5.29 (m)	154.3 (d)	6.93 (d, 15.8)	154.3 (d)	6.95 (d, 15.8)
19	135.0 (s)		70.5 (s)		70.4 (s)	
20	25.7 (q)	1.70 (br s)	29.8 (q)	1.32 (s)	29.7 (q)	1.30 (s)
21	18.1 (q)	1.60 (br s)				
22 (15-Me)	16.8 (q)	1.02 (d, 6.9)	16.9 (q)	1.04 (d, 6.9)	17.0 (q)	1.03 (d, 6.9)
23 (11-Me)	16.3 (q)	1.70 (br s)	16.3 (q)	1.70 (br s)	16.5 (q)	1.68 (br s)

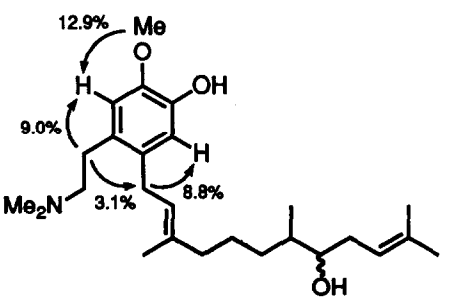
a) ^{13}C NMR spectra were taken at 67.8 MHz. ^1H NMR spectra were taken at 270 MHz.
b) These protons were exchanged gradually with deuteriums of the solvent.

experiment (the percentage of enhancement is shown in D). These units A-G account for all the carbons, hydrogens, oxygens, and a nitrogen, except for a bromine atom in **1**.



The connectivities of these partial structures A-G could be clarified from the COLOC spectrum of **1**: the cross-peaks were observed for H-22 (δ 1.02)/C-14 (δ 33.2), H-22 (δ 1.02)/C-16 (δ 211.9), and NMe_2 (δ 2.28)/C-1 (δ 59.2). Thus, the following connectivities were proved: C-14/C-15/C-16 and C-1/NMe_2. The COLOC spectrum of **1** also afforded cross-peaks for H-2 (δ 2.92)/C-3 (δ 129.1); H-2 (δ 2.92)/C-8 (δ 137.8); H-9 (δ 3.34)/C-3 (δ 129.1); H-9 (δ 3.34)/C-8 (δ 137.8); H-7 (δ 6.75)/C-9 (δ 32.6). These observations proved the following connectivities: C-2/C-3/C-8/C-9 and C-7/C-8/C-9. Based on these findings coupled with the fact that **1** contains a saturated ketone group (1705 cm^{-1}, δ_C 211.9) and a bromine atom, the structure of aplaminone (**1**) was elucidated except for the substitution pattern of the benzene ring, which was proved by the NOE experiments for debromodihydroaplaminone (**4**)[6] obtained as a 1:1 diastereomeric mixture by reduction ($LiAlH_4$, THF) of **1**: the percentages of enhancement in the NOE experiment are shown in **4**. Thus, the structure of aplaminone was determined to be **1**.

Neoaplaminone (**2**), $C_{26}H_{40}BrNO_4$, has one more oxygen than aplaminone (**1**); the spectral properties of both compounds are similar to each other. The extensive analysis of the ^1H-^1H COSY spectrum of **2** led to the



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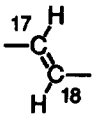
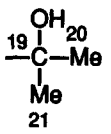
partial structures, H and I, which correspond to the partial structure G in 1. Connection of the partial structures (A-F, H, I, and a bromine atom) by the HMBC spectrum[7] allowed to assign the structure 2 to neoaplaminone. The structure 2 for neoaplaminone was confirmed by the fact that aplaminone (1) was gradually oxidized with molecular oxygen to give neoaplaminone (2).



H



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The molecular formula $C_{26}H_{40}BrNO_7S$ for 3 was established by high resolution negative FABMS and the ^1H and ^{13}C NMR spectral properties of 3 are quite similar to those of 2. Thus, 3 was deduced to be a sulfate of 2. In view of the facts that the proton signal due to H-7 in 3 appeared in the lower field region than the corresponding signal in 2 in their ^1H NMR spectra and that 3 was no longer positive to the $FeCl_3$-$K_3Fe(CN)_6$ test, 3 was deduced to be the phenolic sulfate of 2. This was finally confirmed by the following interconversion between 2 and 3: hydrolysis of 3 (a trace of H_2O, dioxane, 90 °C)[8] provided 2, while sulfation of 2 (1. NaOMe, MeOH; 2. SO_3·Py, CH_2Cl_2) afforded 3. Thus, the structure of neoaplaminone sulfate was elucidated to be 3.

The structures of aplaminone (1), neoaplaminone (2), and neoaplaminone sulfate (3) are novel in that biogenetically they are constructed from a bromine-containing dopamine unit and a sesquiterpenoid part. These compounds, 1, 2, and 3 showed cytotoxic activity against HeLa cells *in vitro*: IC_{50} 0.28, 1.6×10^{-7}, and 0.51 μg/ml, respectively.

References and Notes

1. D. J. Faulkner, *Nat. Prod. Rep.*, **1**, 551 (1984); **3**, 1 (1986); **4**, 639 (1987); **5**, 613 (1988).
2. **1**: $C_{26}H_{40}BrNO_3$ [*m/z* 493.2174 (M^+)]; $[\alpha]^{23}_D$ -2.9° (*c* 1.18, MeOH); UV (MeOH) λ_{max} 224 (ε 11,000), 285 nm (2,900); IR ($CHCl_3$) 3530, 3380 (broad), 1705, 1665, 1595 cm^{-1}; EIMS *m/z* 495 (M^+ + 2), 493 (M^+), 414, 58.
3. **2**: $C_{26}H_{40}BrNO_4$ [*m/z* 509.2142 (M^+)]; $[\alpha]^{23}_D$ -5.3° (*c* 0.65, MeOH); UV (MeOH) λ_{max} 226 (ε 11,000), 284 nm (2,500); IR ($CHCl_3$) 3560, 3400 (broad), 1685, 1660, 1625, 1595 cm^{-1}; EIMS *m/z* 511 (M^+ + 2), 509 (M^+), 493, 491, 430, 58.
4. **3**: $C_{26}H_{40}BrNO_7S$ [*m/z* 588.1642 (M^- - H)]; $[\alpha]^{27}_D$ -3.0° (*c* 1.29, $CHCl_3$); UV (MeOH) λ_{max} 227 (ε 11,000), 282 nm (1,700); IR ($CHCl_3$) 3540 (broad), 1690, 1665, 1630, 1600 (shoulder), 1295, 1045 cm^{-1}; negative FABMS *m/z* 590 (M^- - H + 2), 588 (M^- - H)
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6. Satisfactory IR, ^1H NMR, mass, and exact mass spectral data were obtained for 4.
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