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Aplysepine, a Novel 1,4-Benzodiazepine Alkaloid from the Sea Hare *Aplysia kurodai*

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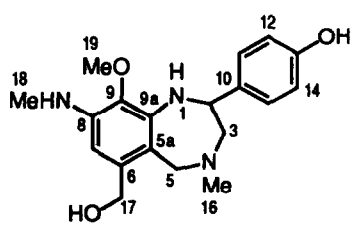
Abstract: Aplysepine (**1**), the first 1,4-benzodiazepine alkaloid of marine origin, was isolated from the sea hare *Aplysia kurodai*. The gross structure of **1** was elucidated on the basis of the spectral data.

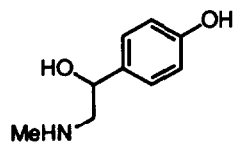
The sea hare *Aplysia kurodai* (Baba) (Aplysiidae) comprises several halogenated terpenoids[1,2] and other unique metabolites.[3–5] In the course of our search for bioactive components of the sea hare,[4,5] we have isolated a novel 1,4-benzodiazepine alkaloid termed aplysepine (**1**). We report herein the isolation and structural elucidation of this compound.

The sea hare *A. kurodai* collected at Azurihama of the Shima Peninsula, Mie Prefecture, Japan, was extracted with MeOH. The EtOAc-soluble material from the methanolic extract was subjected to solvent partitioning (70% MeOH/CH_2Cl_2-CCl_4 1:1) and the 70% MeOH portion was successively chromatographed on alumina and silica gel[6] to afford aplysepine (**1**, 2.4 x 10^{-5}% wet wt) as an amorphous powder.

Aplysepine (**1**), $[\alpha]^{12}_D$ –3.0° (*c* 0.47, MeOH), has a molecular formula of $C_{19}H_{25}N_3O_3$ [high-resolution FABMS: *m/z* 344.1934 (MH^+), Δ –4.0 mmu]. The IR spectrum (3600, 3430, and 3350 cm^{-1}) and the ^1H NMR spectrum in DMSO-d_6 [δ 4.16 (NH), 4.89 (OH), 5.03 (NH), and 9.44 (OH)] indicated the presence of hydroxyl and/or amino groups in **1**. Multiplicities of ^{13}C NMR signals were determined by DEPT experiments and the ^1H-^{13}C COSY spectrum facilitated the assignment of all protonated carbons as shown in Table 1. Because of the presence of many quaternary carbons and hetero atoms, interpretation of the ^1H-^1H COSY spectrum led to only four small segments: –$NHCH_3$, –CH_2OH, –CH_2-CH<, and a 1,4-disubstituted benzene. To assign seven olefinic quaternary carbons (C-5a, 6, 8, 9, 9a, 10, and 13) and connect the segments described above through the quaternary carbons and hetero atoms, COLOC experiments[7] (Table 1) were performed, which allowed to elucidate that **1** is 6-hydroxymethyl-2-(4-hydroxyphenyl)-9-methoxy-4-methyl-8-methylamino-2,3,4,5-tetrahydro-1*H*-1,4-benzodiazepine. The difference NOE experiments of **1**



1



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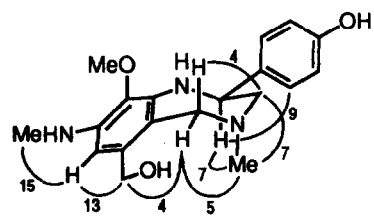

Figure 1. NOE's in %.

Table 1. NMR Spectral Data of **1**.[a]

Psition	^1H[b]	^{13}C	COLOC[c]	position	^1H[b]	^{13}C	COLOC[c]
1	4.16 s	–		10	–	133.7 s	H-1, 2, 12, 14
2	3.19 br d (10.6)	58.5 d	H-11, 15	11, 15	7.23 d (8.4)	127.7 d	H-2
3	2.78 m	66.7 t	H-1, 5 (δ 3.95), 16	12, 14	6.77 d (8.4)	115.5	
5	3.47 d (14.3)	53.6 t	H-16	13	–	156.9	H-11, 15
	3.95 d (14.3)			16	2.27 s	42.3 q	
5a	–	116.6 s	H-1, 5, 7, 17	17	4.41 d (12.8)	62.2 t	H-7
6	–	136.4	H-5		4.48 d (12.8)		
7	6.22 s	103.8 d	H-17, 8-NH	18	2.71 d (5.3)	30.1 q	
8	–	140.5 s	H-18	19	3.47 s	59.3 q	
8-NH	5.03 q (5.3)	–		11-OH	4.89 br s	–	
9	–	135.5 s	H-1, 7, 19	4'-OH	9.44 br s	–	
9a	–	142.1	H-1, 5 (δ 3.95)				

[a] Spectra were recorded at 270 MHz for ^1H and at 67.8 MHz for ^{13}C using DMSO-d_6 as solvent and TMS as internal standard. Chemical shifts are in δ values. [b] Coupling constants in Hz are in parenthesis. [c] Parameters were optimized for J_{CH} = 8 and 6 Hz. Underlined correlations were observed in CD_3OD solution.

not only confirmed the gross structure, but also revealed that the seven-membered heterocyclic ring system of **1** adopted the conformation indicated in Figure 1.

Because of the small specific rotation value ($[\alpha]^{12}_D$ –3.0°) of **1**, we examined the optical purity of **1** by utilizing a chiral HPLC column,[8] revealing the enantiomeric excess of **1** to be 2.4% (the absolute stereochemistry remained unknown).

Although a number of 1,4-benzodiazepines have been synthesized and widely used as anxiolytics and hypnotics, natural 1,4-benzodiazepines (e.g., cyclopenine)[9] are a small group produced by some microorganisms[10] and **1** is the first example of marine origin. A remarkable structural feature of **1** is the substitution pattern of the diazepine ring system which is different from that of other natural 1,4-benzodiazepines: **1** possesses a substituent at C-2, while the others are substituted at C-3.[11] It seems likely that **1** is produced from anthranilic acid and a β-phenethylamine, e.g, synephrine (**2**),[12] whereas natural 1,4-benzodiazepines are known to be biosynthesized from anthranilic acid and α-amino acids via seven-membered cyclic dipeptides.[10]

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