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Aplysidine, a New Nucleoside from the Okinawan Marine Sponge *Aplysina* Sp.

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Abstract: A new nucleoside, aplysidine (**1**), has been isolated from the Okinawan marine sponge *Aplysina* sp. and the structure elucidated by spectroscopic data and confirmed by synthesis of **1**. Synthetically known 2',3'-didehydro-2',3'-dideoxyuridine (**2**) has also been isolated from the same sponge for the first time as a natural product.

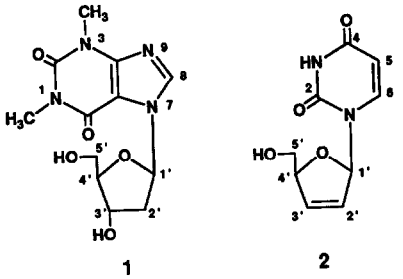
Several biologically active nucleosides have been isolated from marine organisms such as sponges,[1] a gorgonian,[2] nudibranchs,[3] a seaweed,[4] an acorn worm,[5] and a tunicate.[6] During our studies on bioactive substances from Okinawan marine organisms,[7] we have isolated a new nucleoside, aplysidine (**1**), together with the synthetically known 2',3'-didehydro-2',3'-dideoxyuridine (**2**) from the Okinawan marine sponge *Aplysina* sp. In this paper we describe the isolation, structure elucidation and synthesis of **1**.

The sponge *Aplysina* sp. was collected off Kerama Islands, Okinawa, and kept frozen until used. The residue from the methanol extract was partitioned between ethyl acetate and water, and the aqueous portion was subsequently extracted with *n*-butanol. The *n*-butanol soluble material was subjected to a silica gel column ($CHCl_3$/MeOH, 85:15) followed by reversed-phase HPLC on ODS (H_2O/CH_3CN, 90:10) to give aplysidine (**1**, 0.0003 %, wet weight), together with 2',3'-didehydro-2',3'-dideoxyuridine (**2**, 0.001 %).

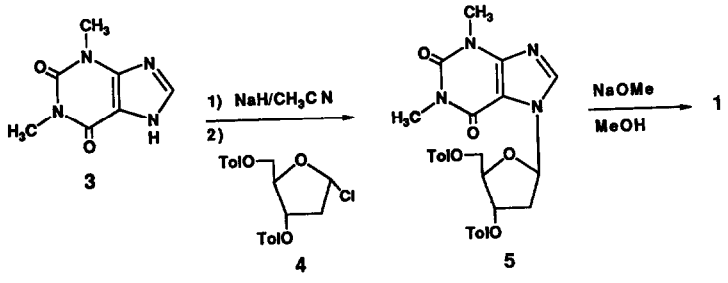
The HREIMS data of **1** established the molecular formula, $C_{12}H_{16}N_4O_5$ (*m/z* 296.1118, M^+, Δ -0.2 mmu), which was supported by the ^{13}C NMR spectrum showing signals for twelve carbon atoms. The IR spectrum indicated the presence of hydroxyl (3360 cm^{-1}) and amide carbonyl groups (1660 cm^{-1}). Nucleoside nature of **1** was indicated by the fragment ion peaks observed at *m/z* 181, 180, and 117 in the EIMS spectrum, which corresponded to $[aglycon + 2H]^+$, $[aglycon + H]^+$, and $[deoxysugar]^+$, respectively.[8] The UV absorption [λmax (H_2O) 274 nm (ε 8500)][9] and ^{13}C NMR signals (δ_C 154.2s, 150.9s, 148.7s, 139.9d, 105.5s, 29.4q, and 27.6q)[10] suggested that **1** possessed 1,3-dimethyl xanthine moiety. The ^1H NMR spectrum showed a singlet of low field resonance [δ_H 8.38 (s)] due to the H-8 of the xanthine ring. Singlets at δ 3.23 and 3.43 were assigned to N-1 and N-3-methyl protons of 1,3-dimethyl xanthine by comparison of the ^1H NMR spectral data with published values.[9] The ^1H NMR signals for the deoxysugar unit were firmly assigned with the aid of decoupling experiments, which established the proton connectivities from H-1' to H_2-5'. Upon addition of D_2O the signals due to H-3' and H_2-5' were simplified and two hydroxyl protons [δ_H

Table 1. ^1H and ^{13}C NMR Data of **1** in DMSO-d_6

position	^1H	J (Hz)	^{13}C
2			150.9 s
4			148.7 s
5			105.5 s
6			154.2 s
8	8.38 s		139.9 d
1'	6.49 t	6.4	85.8 d
2'	2.35 ddd	13.2, 6.4, 4.4	41.3 t
	2.41 ddd	13.2, 6.4, 5.9	
3'	4.31 m		69.7 d
4'	3.86 dt	3.9, 4.4	88.0 d
5'	3.53 dt	11.7, 4.4	61.0 t
	3.61 dt	11.7, 4.4	
N1-Me	3.23 s		27.6 q
N3-Me	3.43 s		29.4 q
3'-OH	5.30 d	3.9	
5'-OH	4.97 brt	4.4	



1 2



3 4 5

5.30 (d, 3'-OH) and 4.97 (brt, 5'-OH)] disappeared. These data suggested that **1** contained 2-deoxyribofuranose portion. The signal of H-1' was observed as a pseudotriplet, indicating a β configuration at the anomeric carbon.[11] Since no NOE was detected for H-1' proton on irradiation of N-3-methyl protons, we assumed the glycosylation site as N-7 and assigned the structure of **1**, therefore, to be theophylline N-7-2-deoxyribofuranoside. This structure was unambiguously confirmed by synthesis of **1** as follows. Treatment[12] of the sodium salt of theophylline (**3**) with 1-chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranose (**4**)[13] in acetonitrile gave 7-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)theophylline (**5**). **5** was treated with sodium methoxide in methanol to give compound **1** (70 % overall yield) whose spectral data were all identical to those of the natural product (**1**). Thus the structure of **1** was concluded to be 7-(2-deoxy-β-D-*erythro*-pentofuranosyl)theophylline.

The molecular formula of **2** was determined as $C_9H_{10}N_2O_4$ by the HREIMS (*m/z* 210.0627, M^+, Δ -1.3 mmu). The ^1H and ^{13}C NMR spectra of **2** showed the presence of a uracil[14] and a 2,3-didehydro-2,3-dideoxyribose group. Compound **2** was thus suggested to be 2',3'-didehydro-2',3'-dideoxyuridine, which was previously known as a synthetic compound.[15] The mp, $[\alpha]_D$, UV, and ^1H NMR of **2** proved to be identical with those of literature.

Aplysidine (**1**) is the first example of nucleoside with a theophylline ring as a base from natural source. Xanthine N-7-ribosides are known to have exhibited antagonistic activity to adenosine A_1 receptor.[16] We found that compound **1** also shows a comparable activity to that of theophylline N-7-riboside.[17] Though compound **2** was previously reported as a synthetic product,[15] the full characterization of **2** has never been reported and this is the first isolation of **2** from natural origin. Nucleosides with 2,3-didehydro-2,3-dideoxyribose have never been isolated from natural sources and it should be noted that this sugar moiety is found in a new anti-HIV drug, 2',3'-didehydro-2',3'-dideoxythymidine (D4T).[18]

EXPERIMENTAL[19]

Isolation. The sponge *Aplysina* sp. [order Verongida, family Aplysinidae] was collected off Kerama Islands, Okinawa, and kept frozen until used. The sponge (1 kg, wet weight) was extracted with methanol (1 L x 2). After evaporation under reduced pressure, the residue (45.0 g) was partitioned between ethyl acetate (500 mL x 3) and 1N NaCl aqueous solution (500 mL). The aqueous layer was extracted with *n*-butanol (500 mL x 3). The *n*-butanol soluble material (4.2 g) was subjected to a silica gel column (4.3 x 34 cm) with $CHCl_3$/MeOH (85:15). The fraction eluted from 500 mL to 720 mL was subjected to reversed-phase HPLC [YMC-Pack ODS-AM323 S-5 120A, 10 x 250 mm; flow rate, 2.5 mL/min; UV detection at 270 nm; eluent, H_2O/CH_3CN (90:10)] to give **1** (2.6 mg, 0.0003 % wet weight; t_R, 25.4 min) and **2** (10.8 mg, 0.001 %; t_R, 9.0 min).

Aplysidine (1). Colorless solid; mp 162°C; $[\alpha]_D^{20}$ +17° (*c* 0.4, H_2O); UV λ_{max} (H_2O) 274 nm (ε 8500), (0.01N HCl) 275 nm (ε 8400), and (0.01N NaOH) 274 nm (ε 8400); IR (KBr) ν_{max} 3360, 3100, 2920, 1705, 1660, 1540, 1410, 1225, 1090, and 745 cm^{-1}; ^1H and ^{13}C NMR (Table 1); EIMS *m/z* 296 (M^+), 181, 180, and 117; HREIMS *m/z* 296.1118 (M^+), calcd for $C_{12}H_{16}N_4O_5$, 296.1120.

2',3'-Didehydro-2',3'-dideoxyuridine (2). Colorless solid; mp 152°C (lit.[15a] mp 153-154°C); $[\alpha]_D^{21}$ -89° (*c* 1.5, H_2O) (lit.[15a] $[\alpha]_D^{24}$ -84° (*c* 0.3, H_2O)); UV λmax (H_2O) 260 nm (ε 10000) (lit.[15a] 261 nm (ε 10380)), (0.01N HCl) 260 nm (ε 10000), and (0.01N NaOH) 260 nm (ε 7000); IR (KBr) ν_{max} 3440, 3170, 3040, 1700, 1670, 1450, 1390, 1250, 1105, and 850 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.58 (2H, brd, *J* = 2.9 Hz), 4.78 (1H, ddt, *J* = 2.9, 2.4, and 1.5 Hz), 4.98(1H, brs), 5.59 (1H, d, *J* = 8.1Hz), 5.92 (1H, ddd, *J* = 5.9, 2.4, and 1.5 Hz), 6.40 (1H, ddd, *J* = 5.9, 3.4, and 1.5 Hz), 6.81 (1H, dt, *J* = 3.4 and 1.5 Hz), 7.74 (1H, d, *J* = 8.1 Hz), and 11.25 (1H, brs); ^{13}C NMR (DMSO-d_6) δ 62.2t, 87.4d, 89.1d, 101.5d, 125.7d, 135.1d, 141.1d, 150.8s, and 163.2s; EIMS *m/z* 210 (M^+), 113, 112, and 99; HREIMS *m/z* 210.0627 (M^+), calcd for $C_9H_{10}N_2O_4$, 210.0640.

7-(2-Deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)theophylline (5). To a suspension of theophylline (**3**; 1.80 g, 10 mmol) in dry acetonitrile (100 mL) was added sodium hydride (60% in oil, 0.48 g, 12 mmol) and the mixture was stirred for 30 min at room temperature under argon atmosphere. Then 1-chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranose (**4**; 3.89 g, 10 mmol) was added and the mixture was stirred for 10 min at room temperature under argon atmosphere. After evaporation under reduced pressure, the residue was suspended in hot ethyl acetate (300 mL) and insoluble materials were removed by filtration. Concentration of the filtrate afforded crystals, which were washed with cold ethyl acetate (2 x 30 mL), and recrystallized from ethyl acetate to yield **5** (3.93 g, 74 %): colorless needles; mp 192°C; $[\alpha]_D^{23}$ -7° (*c* 0.4, H_2O); UV λ_{max} (MeOH) 239 nm (ε 36000) and 272 nm (ε 11000); IR (KBr) ν_{max} 1720, 1700, 1660,

1610, 1540, 1420, 1270, 1100, and 750 cm^{-1}; ^1H-NMR (DMSO-d_6) δ 2.36 and 2.40 (6H, s, toluoyl methyls), 2.80 (1H, m), 3.01 (1H, m), 3.23 (3H, s), 3.41 (3H, s), 4.50 - 4.65 (3H, m), 5.69 (1H, m), 6.63 (1H, t, J = 6.9 Hz), 7.29, 7.37, 7.82, 7.94 (8H, d, J = 8.1 Hz, toluoyl aromatic protons), and 8.37 (1H, s); EIMS *m/z* 532 (M^+), 353, 181, 180, 119, 91, and 81; *Anal.* Calcd for $C_{28}H_{28}N_4O_7$: C, 63.15; H, 5.30; N, 10.52. Found: C, 63.22; H, 5.35; N, 10.88.

Conversion of Compound 5 to Compound 1. To a suspension of **5** (3.46 g, 6.50 mmol) in methanol was added sodium methoxide (28 % in methanol, 1.3 mL, 15.6 mmol) and the mixture was stirred for 2 h at room temperature under argon atmosphere. The reaction mixture was evaporated to dryness, and the residue was passed through a silica gel column (2.3 x 36 cm) with $CHCl_3$/MeOH (88:12). Crystallization from iso-PrOH/MeOH (1:1) yielded compound **1** (1.82 g, 94 %): colorless needles; mp 166°C; $[\alpha]_D^{21}$ +8° (*c* 0.4, H_2O); *Anal.* Calcd for $C_{12}H_{16}N_4O_5$: C, 48.65; H, 5.44; N, 18.91. Found: C, 48.93; H, 5.37; N, 19.14. The spectral data were completely identical with those of the natural product.

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REFERENCES AND NOTES

1. (a) Quinn, R. J.; Gregson, R. P.; Cook, A. F.; Bartlett, R. T. *Tetrahedron Lett.* **1980**, *21*, 567-568. (b) Kato, Y.; Fusetani, N.; Matsunaga, S.; Hashimoto, K. *Tetrahedron Lett.* **1985**, *26*, 3483-3486. (c) Zabriskie, T. M.; Ireland, C. M. *J. Nat. Prod.* **1989**, *52*, 1353-56.
2. Cimino, G.; De Rosa, S.; De Stefano, S. *Experientia* **1984**, *40*, 339-340.
3. (a) Fuhrman, F. A.; Fuhrman, G. J.; Kim, Y. H.; Pavelka, L. A.; Mosher, H. S. *Science* **1980**, *207*, 193-95. (b) Fuhrman, F. A.; Fuhrman, G. J.; Nachman, R. J.; Mosher, H. S. *Science* **1981**, *212*, 557-558.
4. Kazlauskas, R.; Murphy, P. T.; Wells, R. J.; Baird-Lambert, J. A.; Jamieson, D. D. *Aust. J. Chem.* **1983**, *36*, 165-170.
5. Sakemi, S.; Higa, T. *Comp. Biochem. Physiol.* **1985**, *82B*, 107-109.
6. Dematté, N.; Guerriero, A.; Lafargue, F.; Pietra, F. *Comp. Biochem. Physiol.* **1986**, *84B*, 11-13.
7. (a) Kondo, K.; Shigemori, H.; Kikuchi, Y.; Ishibashi, M.; Sasaki, T.; Kobayashi, J. *J. Org. Chem.* **1992**, *57*, 2480. (b) Ishida, K.; Ishibashi, M.; Shigemori, H.; Sasaki, T.; Kobayashi, J. *Chem. Pharm. Bull.* **1992**, *40*, 766. (c) Kobayashi, J.; Takeuchi, S.; Ishibashi, M.; Shigemori, H.; Sasaki, T. *Tetrahedron Lett.* **1992**, *33*, 2579. (d) Tsuda, M.; Shigemori, H.; Ishibashi, M.; Kobayashi, J. *Tetrahedron Lett.* **1992**, *33*, 2597.
8. Biemann, K.; McCloskey, J. A. *J. Am. Chem. Soc.* **1962**, *84*, 2005-2007.
9. Fujii, T.; Saito, T.; Tamura, K. *Chem. Pharm. Bull.* **1991**, *39*, 2855-2862.
10. Claude, N.; Knut, H. *Z. Naturforsch., Teil C* **1974**, *29*, 475-478.
11. Robins, M. J.; Robins, R. K. *J. Am. Chem. Soc.* **1965**, *87*, 4934-4940.
12. Kazimierczuk, Z.; Cottam, H. B.; Revasnkar, G. R.; Robins, R. K. *J. Am. Chem. Soc.* **1984**, *106*, 6379-6382.
13. Hoffer, M. *Chem. Ber.* **1960**, *93*, 2777-2781.
14. Jones, A. J.; Grant, D. M.; Winkley, M. W.; Robins, R. K. *J. Am. Chem. Soc.* **1970**, *92*, 4079-4087.
15. (a) Horwitz, J. P.; Chua, J.; DaRooge, M. A.; Noel, M.; Klundt, I. L. *J. Org. Chem.* **1966**, *31*, 205-211. (b) Lin, T.-S.; Chen, M. S.; McLaren, C.; Gao, Y.-S.; Ghazzouli, I.; Prusoff, W. H. *J. Med. Chem.* **1987**, *30*, 440-444.
16. van Galen, P. J. M.; IJmann, A. P.; Soudijn, W. *Nucleosides and Nucleotides* **1990**, *8*, 275-291.
17. unpublished data
18. Mitsuya, H.; Yarchoan, R.; Broder, S. *Science* **1990**, *249*, 1533-1544.
19. General Procedures are the same as described in the previous papers.[7]