Communications to the Editor

9-Deazaadenosine and Its 5'-α-D-Glucopyranoside Isolated from the Cyanobacterium *Anabaena affinis* Strain VS-1

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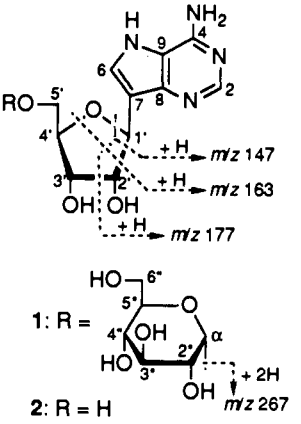
Numerous nucleoside analogs have been synthesized and isolated from natural sources, and their biological activities have also been extensively investigated. While it would be almost impossible to isolate a new type of base per se since synthetic efforts have effectively provided examples of most variations on the base unit,[2] it would be important to isolate as a natural product a base unit which had previously been obtained only by chemical synthesis (nature mimics man!).

In the course of our systematic screening for antiviral, antifungal, antibacterial, and cytotoxic compounds from cyanobacteria (blue-green algae), we found that a MeOH extract of *Anabaena affinis* strain VS-1 showed strong cytotoxicity to L1210 murine leukemia cells, and we assign here structures to two compounds, **1** and **2**, responsible for the cytotoxicity of the organism. This is the first report of the isolation and characterization of pyrrolo[3,2-*d*]pyrimidine derivatives as biosynthetic products.[3]

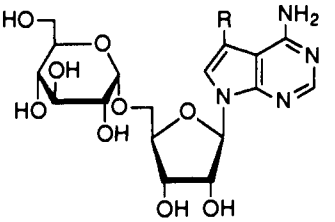
A. affinis strain VS-1 was isolated from a cyanobacterial water-bloom collected from Star Lake, Norwich, VT[4] and cultivated in Z-8 mineral medium according to the conditions reported by Carmichael.[5] The lyophilized cells were extracted with MeOH–H_2O (4:1), and the aqueous residue obtained after evaporation of the MeOH was passed through a CHP-20P column. The column was rinsed with H_2O, and the active components were eluted with 15% EtOH–H_2O and evaporated. Two active components, **1** (0.25% of dried cell weight) and **2** (0.028%), were isolated by bioassay-guided separation of the residue using HPLC with an ODS column.[6]

Compound **1**, $[\alpha]^{28}_D$ +21.9° (*c* 0.051, H_2O), showed a molecular ion peak at *m/z* 429.1627 ($C_{17}H_{24}N_4O_9$, M + H, Δ −0.5 mDa) in the high-resolution (HR) FAB mass spectrum obtained with dithiothreitol/dithioerythritol (magic bullet)[7] as matrix. The ^{1}H NMR spectrum of **1** contained two aromatic proton signals and 13 one-proton signals ascribable to a pentose and a hexose.[8]

Scheme I



Scheme II



Six heteroatom-substituted aromatic ^{13}C signals were detected in the ^{13}C NMR spectrum of **1**, together with 11 signals due to the sugar units.[9] These data and the UV spectrum of **1** [λ_{max} (H_2O) 287 (sh), 276, 268, and 229 nm; (0.01 N HCl) 272 and 235 nm] suggested that **1** is a nucleoside with two sugar units.

Collisionally induced tandem FABMS (FABMS/CID/MS) of **1** showed three major fragment ion peaks at *m/z* 177, 163, and 147 (Scheme I), together with a strong fragment ion peak at *m/z* 267 generated by the loss of the hexose unit, but a prominent peak due to (base + H_2)$^+$ was not detected, suggesting a C-nucleoside.[10] This was confirmed by the chemical shifts of the anomeric center (δ_H, 4.87; δ_C, 78.1), which were observed at relatively high fields in the ^{1}H and ^{13}C NMR spectra of **1**.

Subtraction of the sum of the two sugar units from the molecular formula of **1** gave $C_6H_5N_4$ (133 Da) as the base unit. One-bond ^{1}H–^{13}C coupling constants of ^{13}C signals at δ 128.0 ($^1J_{C,H}$ = 190 Hz) and 149.9 ($^1J_{C,H}$ = 207 Hz) were characteristic

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(6) HPLC retention times (min, Nucleosil 7 C_{18}, 10 mm × 250 mm, 2 mL/min) for **1** and **2**, respectively: MeOH–0.5% AcOH (1:9), 11.1 and 13.1; MeOH–0.05% TFA (1:9), 17.8 and 21.3; MeCN–0.1% NH_4OAc (1:20), 15.4 and 20.2. TLC (R_f value, silica gel, 0.25-mm thick) for **1** and **2**, respectively: $CHCl_3$–MeOH–H_2O (26:15:3), 0.18 and 0.63; EtOAc–2-PrOH–H_2O (4:3:2), 0.24 and 0.50; 1-BuOH–AcOH–H_2O (4:1:1), 0.20 and 0.40.

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(8) ^{1}H NMR data (500 MHz, 26 °C) for **1** in DMSO-d_6 (2.49 ppm): δ 8.05 (s, H-2), 7.72 (d, *J* = 1.0 Hz, H-6), 4.87 (d, *J* = 5.0 Hz, H-1′), 4.74 (d, *J* = 3.5 Hz, H-1″), 4.25 (dd, *J* = 5.0, 5.0 Hz, H-2′), 4.06 (dd, *J* = 5.0, 4.5 Hz, H-3′), 3.90 (ddd, *J* = 4.5, 3.5, 3.0 Hz, H-4′), 3.73 (dd, *J* = 11.0, 3.5 Hz, H-5′), 3.59 (dd, *J* = 11.5, 2.0 Hz, H-6″), 3.52 (dd, *J* = 11.0, 3.0 Hz, H-5′), 3.48 (dd, *J* = 9.5, 9.0 Hz, H-3″), 3.43 (dd, *J* = 11.5, 5.5 Hz, H-6″), 3.39 (m, *J* = 9.0, 5.5, 2.0 Hz, H-5″), 3.23 (dd, *J* = 9.5, 3.5 Hz, H-2″), 3.08 (dd, *J* = 9.0, 9.0 Hz, H-4″), all signals for 1 H; assigned by ^{1}H–^{1}H COSY and single-frequency decoupling experiments.

(9) ^{13}C NMR data (125 MHz, 26 °C) for **1** in DMSO-d_6 (39.5 ppm): δ 150.8 (s), 149.9 (d, $^1J_{C,H}$ = 207 Hz, C-2), 144.4 (s), 128.0 (d, $^1J_{C,H}$ = 190 Hz, C-6), 114.2 (s), 114.1 (s), 98.7 (d, C-1″), 81.8 (d, C-4′), 78.1 (d, C-1′), 74.9 (d, C-2′), 73.4 (d, C-3″), 72.8 (d, C-5″), 72.3 (d, C-2″), 70.9 (d, C-3′), 70.1 (d, C-4″), 66.9 (t, C-5′), 61.0 (t, C-6″); assigned by ^{1}H–^{13}C COSY experiment.

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for carbons attached to, respectively, one and two nitrogen atoms.[11] The ^1H signal (δ_H 7.72) for the hydrogen attached to the former carbon (δ_C 128.0) showed long-range coupling (J = 1.0 Hz) to an anomeric proton (δ_H 4.89, H-1'). These data suggested that the base unit is either pyrrolo[2,3-*d*]pyrimidine (i.e., 7-deazaadenine) or pyrrolo[3,2-*d*]pyrimidine (i.e., 9-deazaadenine). The ^{13}C signals due to the aromatic carbons of **1** resemble those reported for 9-deazaadenine derivatives[12] rather than 7-deazaadenine derivatives,[13] although no pyrrolo[3,2-*d*]pyrimidine derivative has been reported from natural sources.[3] UV spectra of **1**, especially the shifts of absorption maxima in acidic solution, were also more like those of 9-deazaadenine[14] than those of tubercidin (7-deazaadenosine).[15] Accordingly, the base unit in **1** is most likely 9-deazaadenine.

The ^{13}C signals assigned to the hexose unit of **1** closely resembled those of methyl α-D-glucopyranoside,[16] suggesting that **1** is the α-D-glucopyranoside of 9-deazaadenosine. 5'-α-D-Glucopyranosides of tubercidin and toyocamycin (**3** and **4**, respectively, Scheme II) have been isolated from cyanobacteria.[17] ^1H and ^{13}C NMR data for the sugar units of **1** were very similar to those for **3** and **4**, except for the signals due to the C-1' position. Moreover, enzymatic deglycosidation of **1** with α-D-glucosidase gave D-glucose and **2**, which was isolated as the minor component (11% of **1**) from the same cyanobacterium.

Compound **2**, $[\alpha]^{28}_D$ -28.4° (*c* 0.016, H_2O), showed a molecular ion peak at *m/z* 267.1090 ($C_{11}H_{15}N_4O_4$, M + H, Δ +0.3 mDa) by HRFABMS. FABMS/CID/MS of **2** gave the same fragment ion peaks at *m/z* 177, 163, and 147 observed for **1** (Scheme I). The ^1H NMR spectrum of **2** showed the signals ascribable to a ribose unit and two aromatic proton signals.[18]

From the results above, the structure of **2** can be assigned as 9-deazaadenosine, which has been synthesized by Lim and Klein as a cytotoxic C-nucleoside isostere of adenosine.[19] The direct comparison of **2** with a synthetic sample of 9-deazaadenosine[20] by HPLC, TLC, and UV spectra confirmed that **2** was identical to synthetic 9-deazaadenosine.[6] ^1H NMR data for natural **2** hydrochloride were also identical with those for synthetic **2** hydrochloride.[21]

Consequently, the structure of **1** was assigned as the 9-deazaadenosine 5'-α-D-glucopyranoside, as shown in Scheme I. Compounds **1** and **2** are pyrrolo[3,2-*d*]pyrimidine derivatives which have not been reported previously as biosynthetic products,[3] i.e., from natural sources. Their biosynthesis will be of considerable interest.

The IC_{50}s of **1** and **2** vs L1210 murine leukemia cells were 0.01 and 0.002 μg/mL, respectively. These compounds also showed lethal toxicity to the aquatic invertebrate *Ceriodaphnia dubia*; the LC_{50}s for acute (48 h) and chronic (7 day) toxicities were, respectively, 0.5 and 0.3 μg/mL for **1** and 0.3 and 0.1 μg/mL for **2**.

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Supplementary Material Available: ^1H NMR, FABMS/CID/MS, and UV spectra of **1** and **2**; ^{13}C NMR, ^1H-^1H COSY, ^1H-^{13}C COSY spectra of **1**; and ^1H NMR spectra of natural and synthetic **2** hydrochloride (12 pages). Ordering information is given on any current masthead page.

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(18) ^1H NMR data (500 MHz, 18 °C) for **2** in DMSO-d_6 (2.49 ppm): δ 11.95 (1 H, br s, NH), 8.17 (1 H, s, H-2), 7.72 (2 H, br s, NH_2), 7.58 (1 H, s, H-6), 4.85 (1 H, d, *J* = 3.1 Hz, OH), 4.77 (1 H, d, *J* = 7.4 Hz, H-1'), 4.22 (1 H, dd, *J* = 7.4, 5.1 Hz, H-2'), 4.14 (1 H, d, *J* = 3.9 Hz, OH), 4.00 (1 H, dd, *J* = 5.1, 2.8 Hz, H-3'), 3.86 (1 H, ddd, *J* = 3.1, 3.1, 2.8 Hz, H-4'), 3.60 (1 H, dd, *J* = 12.0, 3.1 Hz, H-5'), 3.51 (1 H, dd, *J* = 12.0, 3.1 Hz, H-5'); assigned by single-frequency decoupling experiments.
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(20) The synthetic sample of 9-deazaadenosine hydrochloride was provided by Dr. Robert S. Klein, Montefiore Medical Center.
(21) ^1H NMR data (500 MHz, 18 °C) for **2** hydrochloride in DMSO-d_6 (2.49 ppm): δ 12.80 (1 H, s, NH), 9.03 and 8.99 (each 1 H, s, NH_2), 8.50 (1 H, s, H-2), 7.86 (1 H, d, *J* = 1.0 Hz, H-6), 4.86 (1 H, d, *J* = 7.0 Hz, H-1'), 3.97 (1 H, dd, *J* = 7.0, 5.1 Hz, H-3'), 3.94 (1 H, dd, *J* = 5.1, 3.1 Hz, H-2'), 3.87 (1 H, dt, *J* = 3.2, 3.1 Hz, H-4'), 3.62 (2 H, d, *J* = 3.2 Hz, H_2-5').

[C_6H_6 iso-$C_3H_7^+$] and [$C_6H_7^+$ C_3H_6] Ion-Molecule Complexes: Theoretical Calculations

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While arenium ions are known to be intermediates in electrophilic aromatic substitution reactions,[1] the existence of π-complexes between an aromatic group and a cation remains elusive. In the gas phase the intermediacy of π-complexes has often been postulated in the unimolecular fragmentation of aromatic cations,[2-8] but there is as yet no irrefutable evidence for their existence. The existence of ion-molecule complexes [$C_6H_7^+$ alkene] has been proposed from experimental results.[8] In this work we have, for the first time, calculated the energy and structure of π-complexes and ion-molecule complexes involving the benzenium ion and an alkene. This kind of system is a good example of the use of molecular orbital calculations in order to calculate the energy and to study the structure of ion-neutral complexes. This has been recently reviewed.[9]

We have chosen to focus on one model: the complex intermediates presumed to be involved in the unimolecular reaction of metastable[10] protonated isopropylbenzene. It has been pre-

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