ORGANIC LETTERS

2008
Vol. 10, No. 6
1167−1169

Sorangiadenosine, a New Sesquiterpene Adenoside from the Myxobacterium *Sorangium cellulosum*

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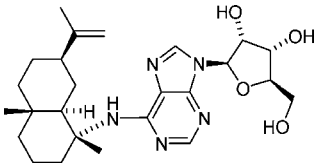
Received January 10, 2008

ABSTRACT



Sorangiadenosine (1), a novel nucleoside possessing a bicyclic, eudesmane-type sesquiterpene as an auxiliary component, was isolated from the culture broth of the gliding bacterium *Sorangium cellulosum* KM1003, collected from Korean soil. The chemical structure of this compound was determined by combined spectroscopic and chemical analyses. The new compound exhibited moderate antibacterial activity against a wide range of bacterial strains.

The myxobacteria produce a wide variety of structurally unique secondary metabolites.[1] Several compounds derived from these organisms exhibit diverse and potent biological activity and are thus attracting a great deal of attention in both biomedicine and synthetic chemistry.[2,3] We have previously reported the chemical structures and bioactivities of bithiazole compounds isolated from *Myxococcus fulvus*.[4,5] In the present study, we utilized a strain of *Sorangium cellulosum* (number KM1003), the crude organic extract of which exhibited significant antibacterial activity toward a series of microbial strains. Here, we describe the chemical structure and bioactivity of sorangiadenosine (**1**), a sesquiterpene adenoside of an unusual structural class, isolated from this crude extract (Figure 1). Isolation and fermentation of microbial strains were carried out according to the procedure reported elsewhere.[6]

The bacterial strain KM1003 was isolated from a soil sample collected from a dry field in Ansan, Korea using WCX solid medium ($CaCl_2 \cdot 2H_2O$ 0.1%, cycloheximide 50 μg/mL, agar 1.5%, pH 7.2). The strain was repeatedly acclimatized using KAN-4 solid medium ($CaCl_2 \cdot 2H_2O$ 0.1%, cycloheximide 50 μg/mL, kanamycin sulfate 0.025%, agar 1.5%, pH 7.2) and maintained in VY/2 solid medium (Baker's yeast 0.5%, $CaCl_2 \cdot 2H_2O$ 0.1%, cyanocobalamin 0.5

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10.1021/ol800061h CCC: $40.75
Published on Web 02/21/2008

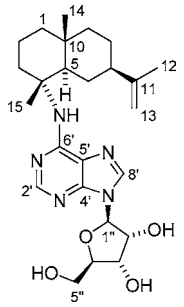

Figure 1. Chemical structures of sorangiadenosine (**1**).

μg/mL, agar 1.5%, pH 7.2), respectively. The strain was identified as *Sorangium cellulosum* by morphological, biochemical, and cultural characteristics.[7,8] The slant culture of KM1003 was cultivated in 2 L Erlenmeyer flasks containing 400 mL of a medium consisting of potato starch 0.8%, glucose 0.2%, soyameal 0.1%, $MgSO_4 \cdot 7H_2O$ 0.1%, $CaCl_2 \cdot 2H_2O$ 0.1%, and EDTA Fe(III)-Na^+ salt 0.0008% in distilled water. Also added to the medium was XAD-16 (1.5% w/v) for the enhancement of metabolite production. Prior to autoclaving, the pH of the medium was adjusted to 7.2 with KOH. The flasks were incubated at 30 °C for 10 days on a rotary shaker at 160 rpm.

At the end of fermentation (20 L), wet cell mass and adsorbent resin XAD-16 were harvested by centrifugation and extracted with acetone (1 L × 3). The aqueous layer, after removal of acetone under vacuum, was adjusted to pH 7 by KOH and twice extracted with EtOAc (1 L × 2).

The organic solution was dried with Na_2SO_4, and the solvent was evaporated under vacuum. The brown residue was separated between 15% aqueous MeOH and *n*-hexane.

Guided by the results of antimicrobial tests and ^{1}H NMR analyses, the aqueous MeOH layer (670 mg) was directly separated by C_{18} reversed-phase HPLC (YMC ODS-A column, 20% aqueous MeOH) to afford pure compound **1** (28.4 mg).

Compound **1** was obtained as a pale-yellow gum, $[\alpha]^{20}_D$ −78.7 ° (*c* 1.17, MeOH). The absorption bands of its IR spectrum suggested the presence of a hydroxyl and/or an amino (3400 cm^{-1}) function groups. The molecular formula $C_{25}H_{38}N_5O_4$ was indicated by the positive HRFABMS at *m*/*z* 472.2922 $[M + H]^+$ (calcd for $C_{25}H_{38}N_5O_4$, 472.2924).

The ^{13}C NMR data of this compound showed signals of 25 carbons; 6 × C, 8 × CH, 8 × CH_2, and 3 × CH_3 (Table 1). A preliminary examination of the ^{1}H, ^{13}C, and ^{1}H COSY NMR data suggested that this compound was composed with three distinct units: a heteroaromatic moiety, a pentose sugar, and a hydrocarbon-type sesquiterpene. Observations of five downfield carbons in the region of δ 155−120 in the ^{13}C NMR spectra and corresponding isolated proton signals at far downfield in the ^{1}H NMR spectra, supported by the presence of several nitrogens in FABMS data, suggested that the heteroaromatic unit of **1** was indeed a base or related moiety. The confirmation of this interpretation and the identification of the base to be adenosine was accomplished by a comparison of the NMR data with those of bases in the literature as well as the *g*HMBC experiment in which several key correlations were found between the protons at δ 8.33 and 8.21 with neighboring carbons.

The presence of a pentose sugar consisted of five characteristic signals in the region of δ 88−60 in the ^{13}C NMR spectra, coupled with the corresponding six proton signals in δ 5.9−3.0 in the ^{1}H NMR spectra, was supported by a combination of ^{1}H COSY and TOCSY experiments in which a linear spin system consisted of all of these protons was traced. The sugar unit was identified as a D-ribose on the basis of acid-hydrolysis followed by GC analysis and

Table 1. NMR Assignments for Compound **1**[a]

no.	δ_H	δ_C	HMBC
1	1.40, m; 1.28, m	40.3 CH_2	
2	1.59, br d (13.8); 1.51, m	18.8 CH_2	C-10
3	2.36, ddd (13.4, 13.1, 4.1); 2.05, br d (13.1)	36.7 CH_2	
4		57.8 C	
5	2.48, dd (12.4, 2.0)	47.7 CH	C-4, C-6, C-7, C-10, C-14, C-15
6	1.57, m; 1.33, m	26.0 CH_2	C-10
7	1.89, br dd (11.9, 11.9)	45.5 CH	
8	1.54, m; 1.49, m	26.7 CH_2	
9	1.40, m; 1.31, m	44.8 CH_2	C-5, C-7, C-10
10		34.2 C	
11		149.9 C	
12	1.64, s	20.6 CH_3	C-7, C-11, C-13
13	4.66, br s; 4.61, br s	108.5 CH_2	C-7, C-11, C-12
14	0.96, s	19.2 CH_3	C-1, C-5, C-9, C-10
15	1.39, s	20.5 CH_3	C-3, C-4, C-5
2′	8.21, s	151.5 CH	C-4′, C-6′
4′		148.0 C	
5′		120.3 C	
6′		154.3 C	
8′	8.33, s	139.6 CH	C-4′, C-5′, C-1″
1″	5.86, d (6.3)	88.0 CH	C-4′, C-8′, C-2″, C-3″, C-4″
2″	5.34, dd (6.3, 5.3)	73.4 CH	C-1″
3"	4.15, dd (5.3, 2.4)	70.6 CH	C-1″, C-5″
4″	3.95, dt (2.4, 2.4)	85.8 CH	C-5″
5″	3.67, dd (12.6, 2.4); 3.54, dd (12.6, 2.4)	61.6 CH_2	C-3″, C-4″
NH	6.28, br s		C-4, C-5, C-10, C-15, C-2′, C-5′, C-6′

[a] Data were obtained in DMSO-d_6 solutions. Assignments were aided by a combination of ^{1}H COSY, TOCSY, *g*HSQC, and *g*HMBC experiments.

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comparison of retention time with authentic pentoses.[9,10] Compound **1** (1.5 mg) was dissolved in MeOH (1 mL) and 0.5 N KOH (0.5 mL) and stirred at room temperature for 2 h. After addition of saturated NaCl (3 mL), the aqueous layer was extracted with EtOAc (3 mL × 3). The EtOAc-soluble fraction contained a mixture of terpene compounds. The aqueous layer was passed through an adsorption column of HP-20 (0.5 × 10 cm) eluted with H_2O (5 mL) and then with MeOH (5 mL). The fraction eluted with MeOH afforded the nucleoside moiety.

The nucleoside moiety (0.7 mg) was dissolved in 0.5 M HCl/MeOH (0.5 mL) and heated at 65 °C for 15 h in a sealed tube. After evaporation of the solvent by a stream of nitrogen, the residue was dissolved in pyridine (50 μL) and treated with hexamethyldisilazane (10 μL) and trimethylsilyl chloride (5 μL) at room temperature for 30 min. Solvent was removed by a nitrogen stream, and the residue dissolved in hexane was analyzed by GC using an HP-1 column (3.2 mm × 50 m). Temperatures of injector and detector were 200 °C for both, and a temperature gradient system was used for the oven. The initial temperature was maintained at 30 °C for 3 min, ramped to 300 °C at the rate of 10 °C/min, then maintained at 300 °C for 3 min. Peak of the hydrolysate was detected at 19.17 min. Retention times for authentic samples after being treated simultaneously with HMDS/TMSCl were 19.17, 19.21 (D- and L-ribofuranose), 18.86, 18.89 (D- and L-lyxofuranose), 18.75, 18.78 (D- and L-arabinofuranose), and 20.07, 20.10 min (D- and L-xylofuranose), respectively. Co-injection of the hydrolysate with the authentic silyated D- and L-ribofuranose enhanced the peak of D-ribofuranose only.

The small vicinal coupling constants (J < 7 Hz) among these protons revealed the furanose nature of this sugar moiety that was secured by *g*HMBC experiments in which a long-range coupling was obtained between the anomeric proton at δ 5.86 and an oxymethine carbon at δ 85.8. In addition, the β-orientation of the anomeric position was assigned on the basis of characteristic $^1J_{CH}$ value (163.3 Hz) as well as the strong enhancement (47%) between H-1 and H-4 in selective-NOE experiment. Thus, the sugar moiety was identified to be a β-D-ribofuranose.[9,11,12]

The sesquiterpene portion of **1** was also determined by a combination of NMR experiments. Signals of protons and carbons of a double bond (δ_C 149.9, 108.5; δ_H 4.66, 4.61) and a vinyl methyl group (δ_C 20.6; δ_H 1.64) in the ^{1}H and ^{13}C NMR data readily revealed the presence of an isopropylene moiety which corresponded to the head of a sesquiterpene. A combination of ^{1}H COSY and TOCSY data revealed several proton spin systems consisted of upfield methylene and methine protons. Long-range correlations of carbons bearing these protons with protons of the terminal isopropylene and two methyls at δ 1.39 and 0.96 in *g*HMBC experiment identified the sequiterpene moiety to be the bicyclic 4-substituted eudesmane (Table 1). The stereochemistry at the asymmetric carbon centers at C-4, C-5, C-7, and C-10 was assigned by NOESY experiments. Cross-peaks at H-2β (δ 1.51)/H-14, H-2β/H-15, H-6β (δ 1.33)/H-8β (δ 1.49), H-6β/H-14, H-6β/H-15, H-8β/H-14, H-14/H-15 which include several 1,3-diaxial correlations of the H-14 and H-15 methyl protons with neighboring protons showed the axial orientations for these. Contrarily, another series of cross-peaks at H-1α (δ 1.28) /H-3α (δ 2.36), H-1/H-5, H-1/H-9α (δ 1.31), H-3/H-5, H-5/H-7, H-5/H-9α showed the axial orientation of these protons at the opposite side of the decalin plane. Thus the ring juncture and configurations were assigned to be trans and 4 *R**, 5 *R**, 7 *R**, 10*R**, respectively.

Finally, the linkage between the adenine and ribose moiety to form an adenosine was achieved by long-range correlations of the H-8′ and H-1″ with carbons in the alternative moiety. Similarly, the linkage between the C-4 of eudesmane and 10-NH of the adenine was secured by long-range correlations of the latter proton with several carbons at the sesquiterpene. Thus, the structure of sorangiadenosine (**1**) was determined to be a new adenosine containing a sesquiterpene of the eudesmane skeleton. Although numerous novel compounds have been isolated from myxobacteria, sorangiadenosine is the first nucleoside containing polyprenyl moiety as an auxiliary unit to the best of our knowledge.

In our measurement of bioactivity against gram-positive and gram-negative bacteria, compound **1** exhibited moderate antibacterial activity with MIC values of 25, 12.5, 6.25, 6.25, 12.5, and >100 μg/mL against the test strains *Staphyloccus aureus* ATCC6538p, *Bacillus subtilis* ATCC 6633, *Micrococcus leuteus* IFC 12708, *Proteus vulgaris* ATCC 3851, *Salmonella typhimurium* ATCC 14028, and *Escherichia coli* ATCC 25922, respectively. Further bioactivity and physiological role of **1** are currently under investigation and will be reported in due course.

Acknowledgment. We express gratitude to Basic Science Research Institute, Seoul, Korea, for providing mass data. K.H.J. and S.-C.C. are the recipients of a fellowship from the Ministry of Education, Korea, through the Brain Korea 21 Project. This work was partially supported by a grant from the Korea Research Foundation (KRF-2006-005-J00502).

Supporting Information Available: Full experimental procedures including spectroscopic and analytical data of compounds along with copies of the ^{1}H NMR, ^{13}C NMR, and 2D NMR spectra of compounds **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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