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SHORT REPORTS

A NUCLEOSIDE DERIVATIVE FROM *EMERICELLA NIDULANS*

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Key Word Index—*Emericella nidulans*; Ascomycotina; fungus; nucleoside; 5′-acetyl-3′-deoxyadenosine; 3′-deoxyadenosine.

Abstract—5′-Acetyl-3′-deoxyadenosine, a new nucleoside derivative, has been isolated from *Emericella nidulans* var. *lata*, together with 3′-deoxyadenosine (cordycepin).

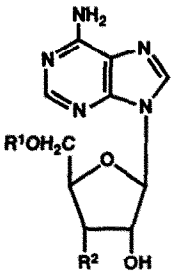
INTRODUCTION

Emericella nidulans (Eidam) Vuill. var. *lata* (Thom & Raper) Subram. (anamorph: *Aspergillus nidulellus* Samson & W. Gams), strain IN-68 is an ascomycetous fungus isolated from the Indonesian medicinal plant, *Trigonella foenum-graecum* L., used as carminatives and tonic medicine [1]. We report the structure of a new nucleoside derivative (**1**) isolated from the ethyl acetate extract of the rice cultures of this fungus, along with a 3′-deoxyadenosine (cordycepin) (**2**) [2, 3].

RESULTS AND DISCUSSION

Compound **1**, gave a molecular ion at *m/z* 293 in the EI mass spectrum, and its elemental analysis confirmed the molecular formula $C_{12}H_{15}N_5O_4$. The strong ion at *m/z* 135 $[C_5H_5N_5]^+$ in the EI mass spectrum and the UV absorption maxima at 258 nm suggested the presence of an adenine moiety in **1**. The ^{1}H NMR signals at δ8.21 (1H, *s*), 8.19 (1H, *s*), and 6.71 (2H, *br s*) were also assignable to aromatic protons at C-2, C-8 and amino group at C-6 of the adenine moiety, respectively. The IR absorption at 1710 cm^{-1} and ^{13}C NMR signals at δ171.5 (*m*), 20.8 (*q*), and ^{1}H NMR signal at δ2.03 (3H, *s*) suggested the presence of an acetoxyl group. The ^{1}H NMR signals at δ6.04 (1H, *d*), 2.14 (1H, *ddd*), and 2.41 (1H, *ddd*) were assigned to protons at C-1′ and C-3′ of the 3′-deoxyribose moiety, respectively. The ^{13}C NMR and IR spectra of **1** were similar to those of 3′-deoxyadenosine (**2**), except the presence of acetoxyl group. Thus the structure of compound **1** was considered to be the monoacetate of compound **2**.

Alkaline hydrolysis of **1** with potassium hydroxide gave compound **2**, which was identical to 3′-deoxyadenosine including the optical rotation. The ^{1}H NMR signals at δ3.52 (1H, *br d*) and 3.71 (1H, *br d*), which were assigned to protons at C-5′ in **2**, shifted downfield to δ4.31 (1H, *dd*) and 4.36 (1H, *dd*) in **1**. This fact suggested that the acetoxyl group is located at C-5′. In order to determine the exact position of the acetate, regioselective acetylation of **2** with *N*,*N*-bis-(2-oxo-3-oxazolidinyl)phosphorodiamidic chloride was examined to give compound **1** [4, 5], which was identical with the naturally occurring 5′-acetyl-3′-deoxyadenosine including the optical rotation. From the above results, the structure of **1** was confirmed as 5′-acetyl-3′-deoxyadenosine.



1 R^1 = Ac, R^2 = H
2 R^1 = H, R^2 = H
3 R^1 = H, R^2 = NHAc

A large number of nucleoside derivatives, which have a wide variety of biological activities, have been isolated from microorganisms. However, only one *N*-acetyl nucleoside derivative has been reported: 3′-acetamide-3′-deoxyadenosine (**3**) was isolated from *Helminthosporium* sp. 215 [6]. 5′-Acetyl-3′-deoxyadenosine (**1**) is the first example of a naturally occurring *O*-acetyl nucleoside.

EXPERIMENTAL

General. Mps: uncorr. LP–LC was performed on a Nihon Seimitsu NP-FX-20 in a glass column (300 × 10 mm) packed with silica gel CQ-3 (30–50 μm; Wako).

Isolation of the metabolites **1** and **2**. *Emericella nidulans* var. *lata* strain IN-68 was cultivated at 25° for 3 weeks on rice (2 kg). The rice cultures were extracted with EtOAc, and the organic layer dried (Na_2SO_4) and evapd *in vacuo*. The residue (5.0 g) was

chromatographed on silica gel with $CHCl_3$–MeOH (20:1) followed by LP-LC using $CHCl_3$–EtOH (10:1) to give 5′-acetyl-3′-deoxyadenosine (**1**) (140 mg), and with $CHCl_3$–MeOH (10:1) to give 3′-deoxyadenosine (cordycepin) (**2**) [1, 2] (mp 225°, 380 mg).

5′-Acetyl-3′-deoxyadenosine (**1**). Needles ($CHCl_3$); mp 166–168°; $[\alpha]_D^{25}$ −21.2° (MeOH; *c* 0.5). IR ν_{max}^{KBr} cm^{-1}: 3450, 3150 (NH_2, OH), 1710 (COO), 1670. UV λ_{max}^{MeOH} nm (log ε): 258 (4.23). EIMS (probe) 70 eV, *m/z* (rel. int.): 293.1117 $[M]^+$ ($C_{12}H_{15}N_5O_4$ requires 293.1123, 3), 234 $[M-MeCO_2]^+$ (5), 164 (59), 135 $[C_5H_5N_5]^+$ (100); (found: C, 49.3; H, 5.3; N, 23.0; calc. for $C_{12}H_{15}N_5O_4 \cdot 1/4\ C_3H_6O$: C, 49.3; H, 5.5; N, 23.0 %); ^1H NMR (300 MHz, acetone-d_6, TMS as int. standard): δ2.03 (3H, *s*, OAc), 2.14 (1H, *ddd*, *J* = 13.3, 5.9, 2.2 Hz, H-3′), 2.41 (1H, *ddd*, *J* = 13.3, 9.7, 5.5 Hz, H-3′), 4.31 (1H, *dd*, *J* = 12.0, 5.0 Hz, H-5′), 4.36 (1H, *dd*, *J* = 12.0, 3.5 Hz, H-5′), 4.69 (1H, *m*, H-4′), 4,88 (1H, *m*, H-2′), 5.13 (1H, *br d*, *J* = 3.1 Hz, OH-2′), 6.04 (1H, *d*, *J* = 1.7 Hz, H-1′), 6.71 (2H, *br s*, NH_2), 8.19 (1H, *s*, H-2 or H-8), 8.21 (1H, *s*, H-2 or H-8); ^{13}C NMR (75 MHz, acetone-d_6, TMS). δ20.8 (*q*, COMe), 35.6 (*t*, C-3′), 66.0 (*t*, C-5′), 76.6 (*d*, C-2′), 79.5 (*d*, C-4′), 93.3 (*d*, C-1′), 121.2 (*s*, C-5), 140.2 (*d*, C-8), 150.8 (*s*, C-4), 154.2 (*d*, C-2), 157.7 (*s*, C-6), 171.5 (*s*, COMe).

Hydrolysis of 5′-acetyl-3′-deoxyadenosine (**1**). A 1 M KOH soln (1 ml) was added to a stirred soln of 5′-acetyl-3′-deoxyadenosine (**1**) (10 mg) in Me_2CO (1 ml). After 1 hr, the reaction mixture was poured into ice-H_2O and extracted with EtOAc. The evapd extract was purified by LP-LC using $CHCl_3$–EtOH (5:1) to obtain 3′-deoxyadenosine (**2**) (4 mg), $[\alpha]_D^{25}$ −38° (H_2O; *c* 0.05). This compound was identical to 3′-deoxyadenosine (**2**) on the basis of a comparison of the ^1H NMR and IR spectra and the optical rotation.

Regioselective acetylation of 3′-deoxyadenosine (**2**). *N,N*-Bis-(2-oxo-3-oxazolidinyl)phosphorodiamidic chloride (150 mg) was dissolved in the mixture of pyridine (1 ml) and triethylamine (2 ml), and HOAc (100 mg). 3′-Deoxyadenosine (**2**) (30 mg) was added to the stirred soln and refluxed for 3 hr. The reaction mixture was poured into ice-H_2O and extracted with EtOAc. The evapd extract was purified by LP-LC using $CHCl_3$–EtOH (10:1) to obtain monoacetate (**1**) (20 mg) as needles ($CHCl_3$); mp 166–168°; $[\alpha]_D^{25}$ −20° (MeOH; *c* 0.1). This compound was identical with naturally occurring 5′-acetyl-3′-deoxyadenosine (**1**) on the basis of a comparison of the ^1H NMR and IR spectra and the optical rotation.

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N^4-METHYLTHERMOSPERMINE IN LEGUMINOUS SEEDS

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Key Word Index—Leguminosae; seeds; polyamines; methylation; methylthermospermine.

Abstract—We found a tertiary methylated tetraamine, N^4-methylthermospermine [$NH_2(CH_2)_3N(CH_3)(CH_2)_3NH(CH_2)_4NH_2$] [PA 3(Me) 34] in addition to spermidine, homospermidine, spermine, thermospermine and canavalmine in extracts of mature seeds of leguminous plants, *Wistaria floribunda*, *Canavalia gladiata* and *Astragalus sinicus* by HPLC and GC-mass spectrometry. This is the first report to show the presence of a tertiary branched methyl derivative of any polyamine.

INTRODUCTION

Leguminous seeds contain various highly basic compounds such as aliphatic polyamines and guanidino compounds. Tetraamines such as spermine (PA 343), thermospermine (PA 334), homospermine (PA 444), aminopropylhomospermidine (PA 344) and canavalmine (PA 434) are distributed in various leguminous seeds [1–6]. Pentaamines such as aminopropylcanavalmine (PA 3434) and aminobutylcanavalmine (PA 4344) were detected in seeds of sword bean *Canavalia gladiata* and pea *Pisum sativum* [2, 4]. A pentaamine, homopentamine