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NUCLEOSIDE ANTIBIOTICS

III. ISOLATION, STRUCTURAL ELUCIDATION AND BIOLOGICAL PROPERTIES OF 3'-ACETAMIDO-3'-DEOXYADENOSINE FROM *HELMINTHOSPORIUM sp.* 215

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SUMMARY

Two additional nucleosides have been isolated from the culture filtrates of *Helminthosporium sp.* 215. They are 3'-acetamido-3'-deoxyadenosine and adenosine. The 3'-amino-3'-deoxyadenosine, isolated from the culture filtrates of Helminthosporium, was converted to 3'-acetamido-3'-deoxyadenosine by treatment with acetic anhydride. The chemical, physical properties and stereochemistry of the synthetic and naturally occurring 3'-acetamido-3'-deoxyadenosine were identical. The mass spectra as reported for 3'-acetamido-3'-deoxyadenosine and 3'-amino-3'-deoxyadenosine are very similar. The acetyl substituent on the 3'-amino-3'-deoxyadenosine position results in a somewhat different fragmentation pattern for 3'-acetamido-3'-deoxyadenosine when compared to 3'-deoxyadenosine. The mass spectrum of 3'-acetamido-3'-deoxyadenosine provides additional evidence for the structure of this nucleoside.

The biochemical properties of 3'-amino-3'-deoxyadenosine and 3'-acetamido-3'-deoxyadenosine are markedly different. 3'-Acetamido-3'-deoxyadenosine was not inhibitory against Ehrlich–Lettre tumor cells nor bacteria while 3'-amino-3'-deoxyadenosine was toxic to ascitic adenocarcinoma. 3'-Acetamido-3'-deoxyadenosine was not toxic to mice, whereas 3'-amino-3'-deoxyadenosine was toxic to mice.

INTRODUCTION

AMMANN AND SAFFERMAN[1] and PUGH, LECHEVALIER AND SOLOTOROVSKY[2] reported that the culture filtrates and mycelial extracts of *Helminthosporium sp.* 215 inhibited the growth of certain tumors. GERBER AND LECHEVALIER[3] subsequently isolated and identified the inhibitor as the nucleoside antibiotic, 3'-amino-3'-deoxyadenosine and reported that this compound inhibited yeast and had antitumor activity. GUARINO AND KREDICH[4] simultaneously isolated the same nucleoside from the mycelia of *Cordyceps militaris*. This nucleoside had been previously synthesized by BAKER, SCHAUB AND KISSMAN[5]. Although puromycin possesses the 3'-amino-3'-deoxyadenosine moiety and is an extremely potent nucleoside analog, 3'-amino-3'-deoxy-

adenosine has no antibacterial activity[3]. CORY AND SUHADOLNIK[6] and FREDERIKSEN[7] reported that 3'-amino-3'-deoxyadenosine was a substrate for adenosine aminohydrolase. 3'-Amino-3'-deoxyadenosine was also shown to be a substrate for partially purified adenosine kinase[8]. SHIGEURA *et al.*[9] and TRUMAN AND KLENOW[10] reported that 3'-amino-3'-deoxyadenosine is an inhibitor of RNA and DNA synthesis in Ehrlich ascites tumor cells. TRUMAN AND KLENOW[10] showed that the DNA dependent RNA polymerase isolated from Ehrlich ascites tumor cells completely inhibited the incorporation of ATP into RNA. KREDICH AND GUARINO[11] and GUARINO AND KREDICH[12] isolated the two additional substituted 3'-amino-3'-deoxyadenosine nucleosides, homocitrullylaminoadenosine and lysylaminoadenosine from mycelial extracts of *C. militaris*.

The purpose of this paper is to describe (a) the isolation of the nucleosides, 3'-acetamido-3'-deoxyadenosine and adenosine from the culture filtrates of *Helminthosporium sp.* 215; (b) the proof of structure, mass spectrum and synthesis of 3'-acetamido-3'-deoxyadenosine; (c) and the effect of 3'-acetamido-3'-deoxyadenosine on tumor cells and microorganisms.

MATERIALS AND METHODS

Isolation of nucleoside antibiotics and adenosine

A culture of *Helminthosporium sp.* 215 was kindly supplied by Dr. NANCY GERBER, Rutgers University, New Brunswick, N.J. The culture medium of GERBER AND LECHEVALIER[3] was used for the production of the nucleoside antibiotics and adenosine. Seven days after inoculation, the mycelium was removed by filtration. The mycelium was washed twice with 100 ml of water. The filtrate and washings were combined. The solution was adjusted to pH 3.4 with 3 M HCl and applied to a Dowex 50(NH_4^+) column (100–200 mesh; 5 cm × 25 cm). The flow rate was 2 l/h. The column was washed with 2.5 l of distilled water (adjusted to pH 3.5 with HCl). The nucleosides were eluted with 2 l of 0.5 M NH_4OH and evaporated to dryness *in vacuo* at 50°–60°. The residue was extracted 3 times by refluxing in 200 ml of methanol for 5 min and filtered. The combined methanol extracts were taken to dryness *in vacuo* at 45° and dissolved in 50 ml methanol–water (1:9, v/v). This solution was added to a Dowex 1(OH^-) column (200–400 mesh; 5 cm × 30 cm)[13]. The column had been previously equilibrated with methanol–water (1:9, v/v). Two 1 l of methanol–water (1:9, v/v), 1 l of methanol–water (25:75, v/v), 1 l of methanol–water (35:65, v/v) and 1 l methanol–water (60:40, v/v) was added. The nucleoside eluted with the 25 % aq. methanol was concentrated to dryness *in vacuo* at 60°. The residue was dissolved in hot water, filtered and crystallized overnight at 4°. The fine, needle-like crystals of 3'-amino-3'-deoxyadenosine were collected by filtration and air-dried. The yield was 105 mg:m.p. 273–275° (decomp.). The nucleoside was shown to be 3'-amino-3'-deoxyadenosine. The nucleoside eluted from the column after treatment with 35 % aq. methanol had a m.p. 262–265°. The yield was 65 mg. This nucleoside was subsequently shown to be 3'-acetamido-3'-deoxyadenosine. The third nucleoside was eluted from the column with 60 % aq. methanol. The yield was 11 mg and the m.p. 229–231°. This nucleoside was subsequently shown to be adenosine.

When NH_4Cl was added to the medium[14], only 3'-amino-3'-deoxyadenosine and adenosine were isolated from the culture filtrates.

Synthesis of 3'-acetamido-3'-deoxyadenosine

3'-Amino-3'-deoxyadenosine (150 mg) was dissolved with warming in 2 ml water and 1.2 ml acetic anhydride followed by a second addition of acetic anhydride (0.8 ml). The solution was shaken for 30 min and cooled at 0°. Excess acetic anhydride was decomposed by the addition of 2 ml of methanol. After 5 min, 3 ml of methanol and 2 ml of water were added and the solution was allowed to stand at room temperature for 15 min. The solution was applied to a Dowex 1(OH^-) column (200–400 mesh; 2.2 cm × 1.5 cm). The acetylated nucleoside was eluted with 500 ml 60 % methanol–water. The solvent was removed by evaporation *in vacuo* below 50°. The residue was dissolved in 30 ml of methanol followed by the addition of 3 ml concentrated NH_4OH. After heating to reflux for 2–3 min, the mixture was allowed to stand for 1 h and evaporated to dryness *in vacuo* below 50°. The residue was dissolved in 8 ml ethanol and filtered. After 2 h at 0°, the crystals were collected by filtration and air-dried; yield 105 mg: m.p. 263–265°.

Paper chromatography

Descending paper chromatography was performed using Whatman No. 1 paper. Four solvents were used: (A) 1-butanol–1 M NH_4OH (86:14, v/v); (B) 1-butanol–formic acid–water (77:10:13, by vol.); (C) ammonia–water (pH 10.3); and (D) 1-butanol–acetic acid–water (5:2:3, by vol.). Nucleosides were visualized on chromatograms under ultraviolet light. Ninhydrin was used for detection of free amino groups.

Instrumental methods

Elemental analyses were performed by Clark Microanalytical Laboratories, Urbana, Ill. Melting points (uncorrected) were taken with a Thomas–Hoover capillary melting point apparatus. Infrared spectra were obtained by 1 % suspensions of nucleosides in KBr pellets with a Perkin–Elmer 337 diffraction-grating spectrometer. Ultraviolet absorption spectra were taken on nucleoside solutions in 0.01 M potassium phosphate, pH 7.0, with a Cary Model 14 recording spectrophotometer. NMR spectroscopy was performed with a Varian HA-60-IL, with benzene as a lock signal and tetramethylsilane as an internal reference. The nucleosides were prepared for NMR analysis by dissolving in 99.5 atom % deuterium oxide followed by evaporation to dryness *in vacuo* below 50°. This process was repeated twice. The sample was then dissolved in 99.5 atom % deuteriated dimethyl sulfoxide (Merck, Sharp and Dohme of Canada). Mass spectra were obtained with a prototype of No. LKB-9000. Bacterial growth was determined with a Klett photoelectric colorimeter.

3'-Amino-3'-deoxyadenosine was a generous gift of Dr. NANCY GERBER, Department of Chemistry, Rutgers University. 3'-Acetamido-3'-deoxyadenosine was a gift of Dr. R. E. SCHAUB, Lederle Laboratories, American Cyanamid Co., Pearl River, N.Y.

Tumor inhibition studies

Groups of ten male Swiss white mice (Huntingdon Farms) were maintained on Purina rat chow and water *ad libitum*. Cages were cleaned daily. Mice were weighed and examined daily. The ascitic form of Ehrlich–Lettre hyperdiploid carcinoma was collected from the peritoneum of mice supplied by Dr. HENRY ALTSCHULER, Albert Einstein Medical Center, Philadelphia, Pa. The cells were centrifuged (1000 × *g*, 20

min, 1°), washed in cold-sterile Krebs–Ringer solution, centrifuged and suspended in sterile physiological saline at a concentration of $5 \cdot 10^7$ cells/ml. Mice were injected intraperitoneally with 0.4 ml of this cell suspension. Control mice received 0.4 ml sterile saline. Sterile solutions of 3'-amino-3'-deoxyadenosine and 3'-acetamido-3'-deoxyadenosine at various concentrations were administered intraperitoneally in 0.5-ml doses to test groups of mice. Control mice received sterile saline.

Inhibition studies

Bacillus cereus and *Bacillus megaterium* were grown at 37° in the following medium: 3.0 g KH_2PO_4, 3.0 g NaCl, 0.7 g glutamic acid, 3.0 g $(NH_4)_2SO_4$, 0.1 g $MgSO_4 \cdot 7H_2O$, 5 mg $FeSO_4 \cdot 7H_2O$, 5 mg $MnCl_2 \cdot 4H_2O$, and 0.5 mg $CuSO_4 \cdot 5H_2O$ in 1 l distilled water. Each flask received a 100 μg amino acid supplement except histidine.

Escherichia coli B was incubated at 30° in the following medium: 7.0 g K_2HPO_4, 3.0 g KH_2PO_4, 0.47 g sodium citrate $\cdot 3H_2O$, 0.1 g $MgSO_4 \cdot 7H_2O$, 1.0 g $(NH_4)_2SO_4$, and 2.0 g glucose in 1 l distilled water.

Streptomyces showdoensis was incubated at 30° in the following medium: 10.0 g glucose, 1.0 g asparagine, 0.1 g K_2HPO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 10 mg $FeSO_4 \cdot 7H_2O$ and 0.5 g yeast extract in 1 l distilled water.

Saccharomyces lindegren (Lindegren breeding strain) was incubated at 30° in Sabouraud glucose medium. All cultures were grown in flasks with Klett tube side-arms. Growth inhibition was determined turbidimetrically. The concentration of 3'-acetamido-3'-deoxyadenosine, 3'-amino-3'-deoxyadenosine or adenosine was 100 μg/ml. The flasks were shaken at 37°.

RESULTS

Isolation of 3'-amino-3'-deoxyadenosine

The use of the technique of DEKKER[13] afforded the isolation of crystalline 3'-amino-3'-deoxyadenosine in yields of 50–60 mg/l. The nucleoside was chromatographically pure (Solvents A, B and C). The R_F values were 0.16, 0.03 and 0.06. These R_F values were the same as those obtained with authentic 3'-amino-3'-deoxyadenosine. The nucleoside areas on the paper chromatograms were detected by ultraviolet light or ninhydrin. The 3'-amino-3'-deoxyadenosine[14] area on the paper chromatograms turned brown after ninhydrin spray treatment and heating at 110° for 5 min.

Isolation and characterization of 3'-acetamido-3'-deoxyadenosine

The nucleoside eluted from Dowex 1(OH^-) with 35 % aq. methanol was judged to be a 9-substituted adenine since it had a sharp maximum at 260 mμ. The $A_{280\ m\mu}/A_{260\ m\mu}$ was 0.16 and the $A_{280\ m\mu}/A_{260\ m\mu}$ for adenosine and 3'-amino-3'-deoxyadenosine were 0.15 and 0.17, respectively. The absorbance at 260 mμ of a 1 % solution of this compound was 506 while that of adenosine was 555. These data suggested that the unknown adenine nucleoside had either a higher molecular weight or lower molar absorbance than that of adenosine.

Paper chromatography of the unknown nucleoside in Solvents A, B and C gave R_F values of 0.28, 0.35 and 0.41, respectively. The R_F values of chemically

TABLE I

PROPERTIES OF CHEMICALLY SYNTHESIZED AND NATURALLY OCCURRING 3'-ACETAMIDO-3'-DEOXYADENOSINE ISOLATED FROM *Helminthosporium sp.* 215

The experimental conditions for the isolation of 3'-acetamido-3'-deoxyadenosine are found under MATERIALS AND METHODS. 3'-Acetamido-3'-deoxyadenosine I, isolated from culture filtrates of *Helminthosporium sp.* 215; II, synthesized by acetylation of 3'-amino-3'-deoxyadenosine that was isolated from culture filtrates of *Helminthosporium sp.* 215; III, this sample of 3'-acetamido-3'-deoxyadenosine was obtained from R. E. SCHAUB.

Properties	*3'-Acetamido-3'-deoxyadenosine*		
	I	*II*	*III*
Ultraviolet spectrum			
λ_{max}, pH 7.0 (mμ)	260	260	260
$A_{280\ m\mu}/A_{260\ m\mu}$	0.16	0.16	0.16
ε_M	15 600	15 800	15 500
Chromatography			
R_F values			
Solvent A	0.28	0.29	0.28
Solvent B	0.35	0.38	0.37
Solvent C	0.41	0.40	0.42
Infrared spectrum			
Carbonyl region (cm^{-1})	1 654	1 651	1 659
NMR spectrum			
3 methyl protons (τ)	8.1	8.1	
Optical rotation			
$[\alpha]_D^{25}$ (0.1 M HCl)	$8\pm2°$	$8\pm1°$	$7\pm3°$*
			$10\pm2°$

* Taken from ref. 5.

synthesized 3'-acetamido-3'-deoxyadenosine in Solvents A, B and C were the same (Table I). No color formation occurred on spraying with ninhydrin. The chromatograms were negative to silver nitrate [15], aminobiphenyl oxalate[16], diphenylamine[17] and thiobarbituate[18]. After treatment of the unknown nucleoside with 1 M HCl in a sealed tube for 1 h at 100° and subsequent paper chromatography (Whatman No. 1, Solvent D), an ultraviolet absorbing spot was detected on the paper chromatogram that had the same mobility as authentic adenine (R_F 0.50). The R_F of the compound before hydrolysis was 0.69. When the unknown nucleoside was treated with 1 M methanolic NaOH at 65° in a sealed tube for 24 h followed by evaporation *in vacuo*, extraction with 95 % ethanol and paper chromatography in Solvent D, an ultraviolet absorbing compound was detected on the paper chromatogram that had the same chromatographic mobility as 3'-amino-3'-deoxyadenosine (R_F 0.54). A control experiment in which 3'-amino-3'-deoxyadenosine was treated identically, showed that under these experimental conditions, 3'-amino-3'-deoxyadenosine was not changed chemically. These data suggest that the 3'-amino-3'-deoxyadenosine moiety was part of the unknown nucleoside. Furthermore, these data also suggest that a substituent was on the 3'-amino group.

The unknown nucleoside was further characterized by infrared, NMR and mass spectroscopy. The infrared spectrum of 3'-amino-3'-deoxyadenosine and the unknown nucleoside were essentially the same except for the strong absorption at

1654 cm^{-1} for the unknown nucleoside. This absorption peak was indicative of a secondary amide. The NMR spectra of deuterated adenosine, 3'-amino-3'-deoxyadenosine and 3'-acetamido-3'-deoxyadenosine were quite similar. A singlet absorption at τ 8.1 (3 H) characteristic of an acetyl methyl group was observed for 3'-acetamido-3'-deoxyadenosine. Additional evidence supporting the idea that this new nucleoside was 3'-acetamido-3'-deoxyadenosine was obtained from elemental analysis. (Found: C, 46.48; H, 5.12; N, 27.54; $C_{12}H_{16}N_6O_4$ requires: C, 46.75; H, 5.25; N, 27.22).

Mass spectra of 3'-amino-3'-deoxyadenosine and 3'-acetamido-3'-deoxyadenosine

The mass spectrum of 3'-acetamido-3'-deoxyadenosine (Fig. 1) differs from that observed for adenosine[19] and 3'-deoxyadenosine[20]. The structural assignment for 3'-acetamido-3'-deoxyadenosine is in part confirmed by the appearance of the

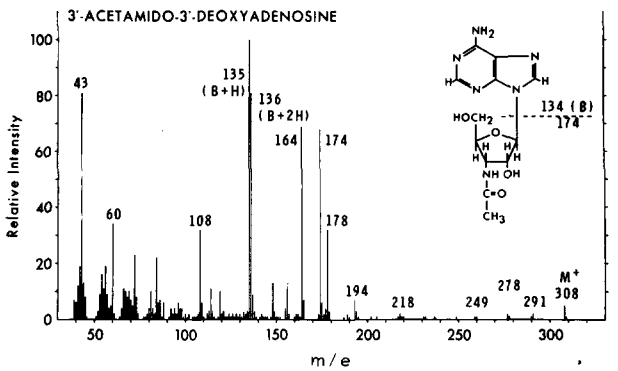


Fig. 1. Mass spectrum of 3'-acetamido-3'-deoxyadenosine (70 eV). This spectrum was computer plotted from tabular intensity data. A CalComp 565 plotter driven by an IBM 1620 computer required about 3 min of plotting time and 6 min of computer time (for a Fortran II-D program).

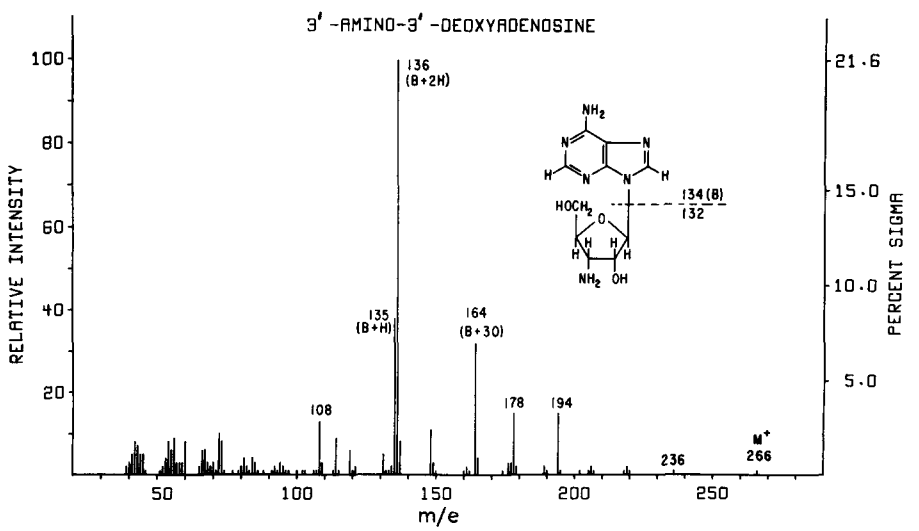
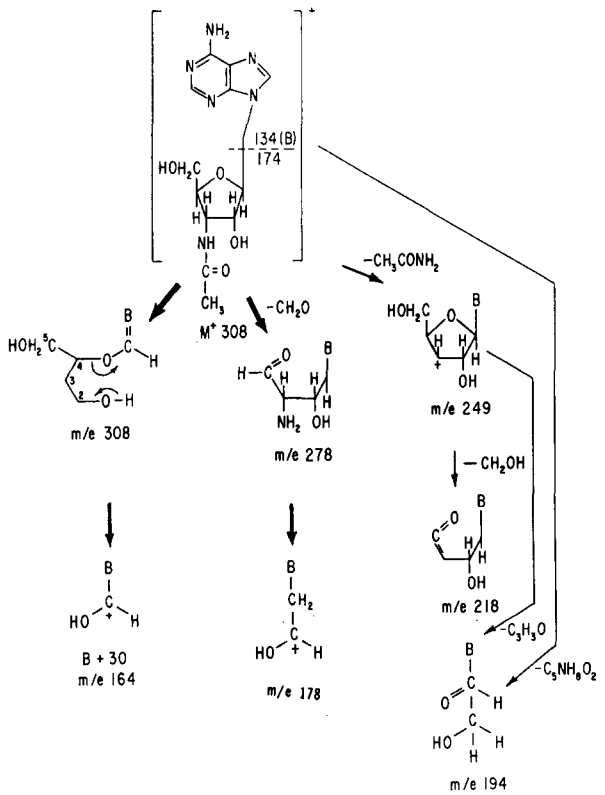


Fig. 2. Mass spectrum of 3'-amino-3'-deoxyadenosine (70 eV). This spectrum was computer plotted from tabular intensity data. A CalComp 565 plotter driven by an IBM 1620 computer required about 3 min of plotting time and 6 min of computer time (for a Fortran II-D program).

molecular ion at *m/e* 308. The loss of the acetamido group (M-59) is shown by *m/e* 249. The hydrogen on carbon atom 4′ is probably transferred to the CH_3CONH group in this transition. Absolute proof for this statement must be obtained through isotope labeling experiments. The ion at *m/e* 60 (acetamide+H^+) and the ion at *m/e* 43 (acetylium ion) are further proof for the structural assignment given 3′-acetamido-3′-deoxyadenosine isolated from the culture filtrates of *Helminthosporium sp.* 215. The ion at *m/e* 60 should be particularly useful in ^{15}N experiments to study the precursor for the amino-nitrogen at the 3′-position of 3′-acetamido-3′-deoxyadenosine.

The B+2H ion (*m/e* 136) intensity for 3′-acetamido-3′-deoxyadenosine (Fig. 1) is less than that observed for 3′-amino-3′-deoxyadenosine (Fig. 2) but more intense than that observed for 3′-deoxyadenosine[20]. This difference is probably attributed to the acetyl substituent on the 3′-amino group of the nucleoside antibiotic. The mass spectra of 3′-acetamido-3′-deoxyadenosine and 3′-amino-3′-deoxyadenosine have three similar peaks at *m/e* 164, 178 and 194. (Scheme 1). Additional fragments observed for 3′-acetamido-3′-deoxyadenosine include the loss of (1) a hydroxyl (M−17, *m/e* 291); (2) formaldehyde (M−30, *m/e* 278); (3) HCN ((B+H)−29, *m/e* 108) and (4) the direct loss of B(M−134,*m/e* 174). The peak for 3-acetamidoribofuranose (*m/e* 174)



PRINCIPLE FRAGMENTATION PATHS OF 3'-ACETAMIDO-3' DEOXYADENOSINE

Scheme I. Principle fragmentation paths of 3′-acetamido-3′-deoxyadenosine.

from 3'-acetamido-3'-deoxyadenosine is probably attributed to the increased stability of 3-acetylaminoribose since 3-aminoribose was not an observed fragment in the mass spectrum of 3'-amino-3'-deoxyadenosine. Further evidence for adenosine in the nucleoside is provided by the loss of HCN from the purine ring. The loss of HCN is similar to reports of RICE AND DUDEK[21], SMULSON AND SUHADOLNIK[22] and EGGERS *et al.*[23] on the mass spectra of adenosine, 7-deazaadenine and puromycin.

The physical properties lending support for the structure of the unknown nucleoside as 3'-acetamido-3'-deoxyadenosine are summarized in Table I.

Characterization of adenosine as the nucleoside eluted by 60 % methanol

The chemical and physical properties of the crystalline compound eluted from the culture filtrates of Helminthosporium were identical with that of authentic adenosine. The m.p. 230°–231°, the infrared spectrum, the ultraviolet spectrum and the NMR spectrum were identical.

Evaluation of 3'-acetamido-3'-deoxyadenosine for toxicity and tumor cell growth inhibition

The toxicities of 3'-amino-3'-deoxyadenosine and 3'-acetamido-3'-deoxyadenosine administered intraperitoneally in mice were markedly different. Mice receiving one injection (11 mg per kg body weight) of 3'-amino-3'-deoxyadenosine lost an average of 13 % body weight but did not die. Mice given a larger dose (23 mg/kg) died within six days. The average loss of body weight was 22 %. The mice receiving 3'-acetamido-3'-deoxyadenosine (one injection, 47 mg/kg) did not die after 15 days nor did they lose weight.

The mean survival time of mice receiving an intraperitoneal injection of Ehrlich–Lettre ascitic tumor cells was six days; the average weight gain was 28 %. Tumor-inoculated animals that received 50 μg of 3'-acetamido-3'-deoxyadenosine had a mean survival time of six days and an average weight gain of 21 %. Those mice treated with tumor cells and 50 μg of 3'-amino-3'-deoxyadenosine survived an average of 17 days with a 25 % increase in body weight after six days and an 80 % increase in body weight at 20 days. These findings with 3'-amino-3'-deoxyadenosine are in agreement with the earlier report of GERBER and LECHEVALIER[3] and the antitumor properties of this nucleoside. 3'-Acetamido-3'-deoxyadenosine did not inhibit the growth of the ascitic form of Ehrlich–Lettre carcinoma.

Effect of 3'-acetamido-3'-deoxyadenosine on the growth of bacteria

3'-Amino-3'-deoxyadenosine and 3'-acetamido-3'-deoxyadenosine did not inhibit the growth of *B. cereus*, *B. megaterium*, *E. coli* B, *S. showdoensis* or *S. lindegren*.

DISCUSSION

By employing the technique for the isolation of nucleosides as described by DEKKER[13], three nucleosides were isolated from the culture filtrates of *Helminthosporium sp.* 215. 3'-Amino-3'-deoxyadenosine had been isolated and reported earlier[3,4]. There was no previous report concerning the occurrence of additional nucleosides in the culture filtrates. This report describes the isolation and identification of the two additional nucleosides from the culture filtrates of Helminthosporium, 3'-acetamido-

3′-deoxyadenosine and adenosine. The amount of adenosine occurring in the culture filtrates is about one-tenth that of 3′-amino-3′-deoxyadenosine and the amount of 3′-acetamido-3′-deoxyadenosine is about one-half that of 3′-amino-3′-deoxyadenosine. The role of 3′-aminonucleosides and the presence of adenosine in the culture filtrate of *Helminthosporium sp.* 215 has not yet been established. The use of mass spectrometry has been especially useful to determine the structural features of nucleosides. The mass spectrometric evidence (Fig. 1) along with the chemical and physical properties described provide unequivocal evidence for the structure of 3′-acetamido-3′-deoxyadenosine isolated from the culture filtrates of *Helminthosporium sp.* 215. Since SUHADOLNIK, WEINBAUM AND MELOCHE[24] reported that adenosine is the precursor for the biosynthesis of 3′-deoxyadenosine (cordycepin), it may be that Helminthosporium synthesizes adenosine in quantities greater than that which can be utilized for the biosynthesis of the 3′-aminonucleosides. DNA synthesis, but not RNA synthesis, ceases when the nucleosides appear in the culture filtrate. Ammonium chloride stops the production of 3′-acetamido-3′-deoxyadenosine while biosynthesis of the nucleosides, 3′-amino-3′-deoxyadenosine and adenosine continues. Growth inhibition studies were performed with 3′-acetamido-3′-deoxyadenosine to determine the effect of the *N*-acetyl group on tumor and bacterial cells. While 3′-amino-3′-deoxyadenosine has marked antimitotic and antitumor activity[1,3] and inhibits RNA and DNA synthesis[9,10], 3′-acetamido-3′-deoxyadenosine did not inhibit the growth of Ehrlich–Lettre tumor cells. This marked difference in the biological properties of 3′-acetamido-3′-deoxyadenosine and 3′-amino-3′-deoxyadenosine in Ehrlich–Lettre tumor cells might be attributed to differences in the deamination, hydrolysis of the N-riboside bond, lack of permeability or a lack of phosphorylation. Of these four possibilities, the latter is the most likely explanation for the lack of inhibition of 3′-acetamido-3′-deoxyadenosine against Ehrlich ascites tumor cells, since SHIGEURA *et al.*[9] concluded that the lack of inhibition of nucleosides was attributed to the complete absence of phosphorylation. They reported that an amino group on C-3′ of adenosine did not affect phosphorylation, while an alkyl group substituted on the 6-amino moiety drastically interfered with phosphorylation of the nucleoside. It may be that the *N*-acetyl substituent of 3′-acetamidc-3′-deoxyadenosine may be bulky enough to prevent phosphorylation. BENNETT and co-workers[25,26], LINDBERG, KLENOW AND HANSEN[8], MITSUGI *et al.*[27], SHIGEURA *et al.*[9], SHIGEURA AND SAMPSON[28] and TRUMAN AND KLENOW[10] have studied the substrate specificity of adenosine kinase and showed that a number of adenosine analogs are substrates. Many of the 5′-monophosphates of the adenosine analogs were converted to the triphosphates[8,10,28]. 3′-Acetamido-3′-deoxyadenosine was not tested as a substrate.

Total deamination and or hydrolysis of the acetamido nucleoside is unlikely, since SHIGEURA *et al.*[9] and TRUMAN AND KLENOW[10] reported that 3′-amino-3′-deoxyadenosine is phosphorylated to the 5′-triphosphate and the N–riboside bond was not hydrolyzed by Ehrlich ascites tumor cells. 3′-Acetamido-3′-deoxyadenosine may also be deaminated by Ehrlich–Lettre tumor cells since CORY AND SUHADOLNIK[6], FREDERIKSEN[7] and CODDINGTON[30] reported that 3′-amino-3′-deoxyadenosine is a good substrate for calf intestinal adenosine deaminase. KLENOW[29] did report that deaminated cordycepin did not inhibit the incorporation of $^{32}P_i$ into the nucleic acids of Ehrlich ascites tumor cells. Apparently, the *N*-acetyl group of 3′-acetamido-3′-deoxyadenosine is not hydrolyzed by Ehrlich ascites tumor cells since removal of this acetyl group

would result in the formation of 3'-amino-3'-deoxyadenosine which is known to inhibit RNA and DNA synthesis in these cells[10,28]. 3'-Amino-3'-deoxyadenosine and 3'-acetamido-3'-deoxyadenosine failed to inhibit the growth of five bacteria.

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