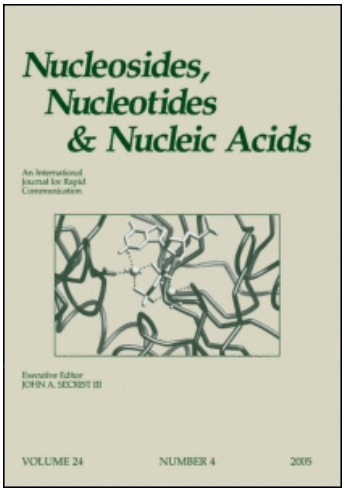

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Biosynthesis of the Naturally Occurring Nucleoside Antibiotics from Adenosine

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BIOSYNTHESIS OF THE NATURALLY OCCURRING NUCLEOSIDE ANTIBIOTICS FROM ADENOSINE

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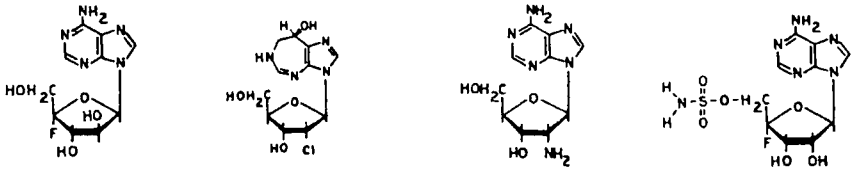
9-β-D-Arabinofuranosyladenine (ara-A, **1**), first isolated from the culture filtrates of Streptomyces antibioticus, has a broad spectrum of activity against DNA viruses in cell culture and is successfully used in therapy of herpes simplex encephalitis, neonatal herpes, herpes zoster and chronic myelogenous leukemia[1]. 2'-Chlorodeoxycoformycin (2'CldCF, **2**), 2'-amino-2'-deoxyadenosine (**3**) and nucleocidin (**4**) have been isolated from the culture medium of Actinomadura and S. clavus, respectively.



1 **2** **3** **4**

[U-^{14}C]Adenosine is the direct carbon-nitrogen precursor for these four naturally occurring nucleoside antibiotics. These data were obtained by the addition of [U-^{14}C]adenosine to nucleoside antibiotic-producing cultures followed by isolation, crystallization to constant specific activity and hydrolysis to their respective aglycones and pentofuranosyl moieties[2-4]. The ratios of ^{14}C in the aglycone: pentofuranosyl moieties of ara-A, 2'CldCF, 2'-amino-2'-deoxyadenosine and nucleocidin are summarized in Table 1. Additional proof that the N-ribosyl bond of the [U-^{14}C]adenosine is not hydrolyzed during uptake followed by an intracellular resynthesis to form AMP was obtained by the addition of unlabeled adenine simultaneously with the [U-^{14}C]adenosine to the

Table 1. Ratios of ^{14}C in Ara-A, 2'CldCF, 2'-Amino-2'-deoxyadenosine and Nucleocidin

Nucleoside	%[a,b,c]
Ara-A isolated from [2'-^{18}O]- and [U-^{14}C]adenosine:	
adenine	56
D-arabinose	44
Ara-A isolated from [3'-^{18}O]- and [U-^{14}C]adenosine:	
adenine	58
D-arabinose	42
2'-Chloro-2'-deoxycoformycin isolated from [U-^{14}C]-adenosine:	
aglycone	47
2'-chloro-2-deoxyribose	53
2'-Amino-2'-deoxyadenosine isolated from [U-^{14}C]-adenosine:	
adenine	46
2-amino-2-deoxy-D-ribofuranose	54
Nucleocidin isolated from [U-^{14}C]adenosine:	
adenine	49
4-fluoro-5-sulfamoyl-D-ribofuranose	51

[a]Ratios of ^{14}C in the adenosine added to the cultures for ara-A, 2'CldCF, 2'-amino-2'-deoxyadenosine and nucleocidin experiments were: 51:49, 48:52, 48:52, and 54:46, respectively.

[b]For the determination of the ^{14}C ratios in the aglycone: pentose moieties of the ara-A, 2'CldCF, 2'-amino-2'-deoxyadenosine and nucleocidin, >5000 dpm of crystalline nucleoside was hydrolyzed; recoveries were 81-95%.

[c]The ^{14}C ratios of the adenosine isolated from the RNA of the organism producing ara-A, 2'CldCF, 2'-amino-2'-deoxyadenosine, and nucleocidin were 57:43, 42:58, 42:58, and 42:58, respectively. Similar ratios of ^{14}C in the aglycone:pentose were obtained in experiments in which unlabeled adenine was added together with the [U-^{14}C]adenosine.

cultures. Isolation of the four nucleoside antibiotics from this experiment showed that the ratios of the ^{14}C in the aglycone:pentose moieties were the same. Further, the ^{14}C ratios in the adenine:ribose of the adenosine isolated from the RNA were essentially the same as the [U-^{14}C]adenosine added to the nucleoside antibiotic-producing cultures (Table 1, footnote c).

To study the chemistry at C-2' and C-3' of adenosine during the biosynthesis of ara-A by S. antibioticus, ^{18}O:^{14}C double label in vivo experiments were performed with S. antibioticus. The [2'-^{18}O]adenosine and [3'-^{18}O]adenosine were synthesized by Baker and coworkers[5]. In double label in vivo experiments with [2'-^{18}O]adenosine (50% ^{18}O enrichment) and [U-^{14}C]adenosine or [3'-^{18}O]adenosine and [U-^{14}C]adenosine, the ^{18}O:^{14}C ratios in the ara-A and adenosine isolated from the RNA were 2.42 and 2.94, respectively. This compares with ^{18}O:^{14}C ratios of 2.37 of the [2'-^{18}O]- and [U-^{14}C]adenosine added to the cultures. However, in experiments with [3'-^{18}O] (48% ^{18}O enrichment) and [U-^{14}C]adenosine, the [U-^{14}C]adenosine was converted to ara-A but there was no detectable ^{18}O at C-3' of ara-A. The ^{18}O:^{14}C ratios for the double label [2'-^{18}O]adenosine and [U-^{14}C]adenosine and the [3'-^{18}O]adenosine and [U-^{14}C]adenosine of the adenosine from the RNA were 2.94 and 3.11, respectively. An enzyme has also been isolated and partially purified from S. antibioticus that catalyzes the conversion of adenosine but not AMP, ADP, ATP, inosine, guanosine nor D-ribose to ara-A. With the partially purified enzyme, with [2'-^{3}H]adenosine, the C-2'-H bond was cleaved as evidenced by the release of 8.9% of the tritium from C-2' as tritium oxide. The enzyme displays saturation kinetics, a pH optimum of 6.8, a K_m of 8×10^{-4} M and is inhibited by heavy metals. Tubercidin, but not sangivamycin, is converted to ara-tubercidin by the enzyme.

With respect to the direct conversion of adenosine (or adenosine nucleotide) to 2'CldCF and 2'-amino-2'-deoxycoformycin, the data show that the hydroxyl at C-2' of the ribosyl moiety of adenosine undergoes a replacement by a chloro or an amino group. Furthermore, [^{36}Cl]chloride when added to the culture medium resulted in the isolation of [^{36}Cl]2'CldCF. Finally, the direct conversion of [U-^{14}C]adenosine to nucleocidin (4) by S. clavus (Table 1) demonstrates that the C-4'-H is stereochemically replaced by fluoride with retention of configuration. The mechanisms for the regioselective modification of the C-2' hydroxyl group and the hydrogen at C-4' of adenosine and the stereospecific insertion of the amino-, chloro-, and fluoro groups are under investigation.

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