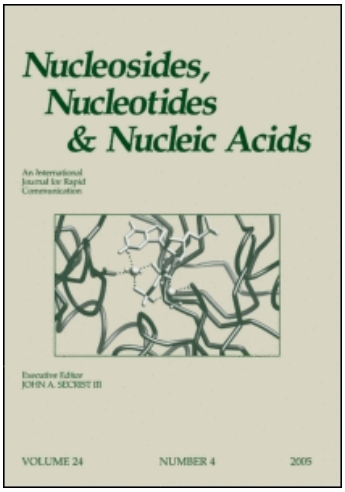

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STUDIES ON THE CHEMICAL SYNTHESIS OF POTENTIAL ANTIMETABOLITES. 36.[1] SYNTHESIS AND SOME BIOCHEMICAL PROPERTIES OF (±)1-DEAZAARISTEROMYCIN

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Abstract — Chemical synthesis of a carbocyclic nucleoside, (±)1-deazaaristeromycin (1), is described. Compound 1 was found to possess only a weak inhibitory effect on calf intestine adenosine deaminase. For rabbit liver S-adenosylhomocysteinase 1 showed weak affinity but acted as a potent irreversible inactivator.

S-Adenosylhomocysteinase (SAHase, EC 3.3.1.1) catalyzes reversible hydrolysis of S-adenosylhomocysteine (SAH) to adenosine and homocysteine. SAH is known to be a potent product inhibitor of S-adenosylmethionine-dependent methylation reactions involving nucleic acids, proteins, and phospholipids. Inhibition of SAHase may lead to an increase of SAH concentration, thereby perturbing *in vivo* methylation reactions.

The involvement of methyl transfer reactions in a variety of biological phenomena[2] points to the possibility that inhibition of these reactions may be responsible for the pharmacological action of certain compounds. For example, it has been known that the antiviral agents 3-deazaadenosine and 9-β-D-arabinofuranosyladenine (AraA) are potent inhibitors of SAHase. Thus, the concept of SAHase as a target for antiviral action of adenosine analogues has recently emerged[2]

The findings that carbocyclic nucleosides (±)3-deazaaristeromycin[3] (2) and neplanocin A[4,5] exhibit potent inhibitory activities for SAHases as well as a broad spectrum of antiviral activities prompt us to prepare an additional carbocyclic analogue, (±)1-deazaaristeromycin (1).

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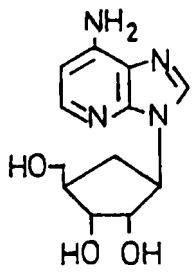

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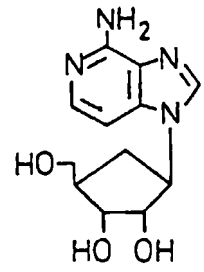

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Synthesis of 1 was performed analogously according to the method for the preparation of 9-hydroxyethyl-1-deazaadenine[6]. Thus, 4-amino-2-chloro-3-nitropyridine[7] (3) was coupled with (±)4β-amino-2α,3α-dihydroxy-1β-cyclopentanemethanol (4a), prepared by a slight modification of Saksena's procedure[8], to give compound 5a in 46 % yield. Catalytic hydrogenation over Raney nickel gave the corresponding triaminopyridine derivative (6a), which was subjected to cyclization with formamidine acetate to afford 1 in 46 % yield based on 5a. The ultraviolet absorption spectrum of 1 was superimposable with 1-deazaadenosine[6,9] and pmr spectral pattern due to the carbocyclic hydrogens was quite similar to that of aristeromycin. The overall yield was, however, comparatively low (4a through to 1, 21 %) because of the difficulty of purification of the intermediates (4a through to 6a in Fig. 1). In order to overcome this problem, an isopropylidene derivative of the cyclopentane methanol (4b) was chosen as an amine component in place of 4a.

A series of reactions (4b through to 7) proceeded much smoothly and 2',3'-O-isopropylidene-(±)1-deazaaristeromycin (7) was converted to 1 by treatment with aqueous formic acid. The overall yield was improved to some extent (29 %).

Preliminary Evaluation of Biochemical Properties

Compound 1 was tested for its ability to inhibit calf intestine adenosine deaminase and rabbit liver SAHase[10]. Compound 1 was found to be a weak competitive inhibitor with a Ki value of 140 μM for the adenosine deaminase[11], while 1-deazaadenosine has been reported to be a competitive inhibitor with a Ki value of 2 μM[11b]. Aristeromycin has been known to act as a substrate with a Km value of 3.3 mM, showing less affinity to the enzyme compared with adenosine (Km 36 μM)[12]. These

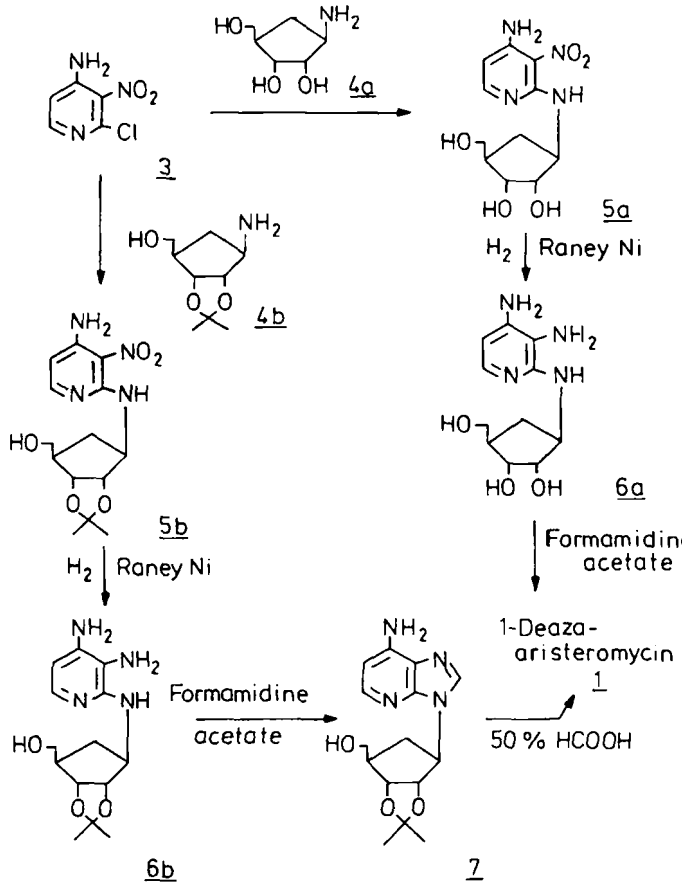

Fig. 1. Preparation of (±)1-Deazaaristeromycin (1)

findings suggest that the replacement of the ribose moiety with a carbocyclic counterpart may weaken the affinity to the adenosine deaminase.

For the rabbit liver SAHase, 1 showed no significant affinity with a Ki value of 530 μM but may act as a potent irreversible inactivator with a k value[13] of 1.1 x 10^4 $M^{-1}min^{-1}$. In contrast, compound 2 has been shown to be a competitive inhibitor with Ki values of 3 μM and 1 nM for the enzyme from calf liver and hamster liver, respectively, but does not inactivate to any extent[3a]. (±)Aristeromycin is reported to act as an inhibitor for calf liver SAHase both in a competitive[14] and an irreversible manner[13b]. It is worthy of note that the isosteric carbocyclic deazanucleosides closely related to aristeromycin exhibit such a sharp dif-

ference in terms of interactions with SAHases. Details of the enzymological results using optically active 1-deazaaristeromycin will be the subject of a forthcoming paper.

EXPERIMENTAL

Melting points were determined with a Yamato melting point apparatus, type, MP-1, and are uncorrected. Ultraviolet absorption spectra were taken on a Hitachi 323 recording spectrophotometer. Pmr spectra were obtained on a JEOL FX-100 and FX-200 spectrometers using tetramethylsilane as an internal standard. Signals are designated as s (singlet), bs (broad singlet), d (doublet), t (triplet), and m (multiplet). Mass spectra were taken on a JEOL JMS-D300 mass spectrometer.

(±)4β-(4-Amino-3-nitro-2-pyridylamino)-2α,3α-dihydroxy-1β-cyclopentane methanol (5a)

A mixture of (±)4β-amino-2α,3α-dihydroxy-1β-cyclopentanemethanol (4a, 163 mg, 1.1 mmol) and 4-amino-2-chloro-3-nitropyridine (3, 210 mg, 1.2 mmol) in absolute nitromethane (5 ml) containing triethylamine (0.3 ml) was refluxed for 5 h. After cooling the mixture to room temperature, precipitated yellow material was filtered and washed with water. Recrystallization from water gave 5a (140 mg, 44 % based on 4a) in a pure state, mp 224° MS (m/z): 284 (M^+), 267, 249. Pmr in DMSO-d_6 (δ in ppm): 1.12 (m, 1H,H-5'a), 1.95 (m, 1H, H-4'), 2.28 (m, 1H, H-5'b), 3.40 (m, 2H, H-6'), 3.72 (m, 2H, H-2' and H-3'), 4.35 (m, 2H, H-1' and OH), 4.64 (t, 1H, OH), 4.87 (m, 1H, OH), 6.10 (d, 1H, H-5, J = 5.9 Hz), 7.63 (d, 1H, H-6, J = 5.9 Hz), 8.13 (bs, 2H, NH_2), 8.80 (bd, 1H, NH).

Anal. Calcd for $C_{11}H_{16}N_4O_5$: C, 46.48; H, 5.67; N, 19.71. Found: C, 46.30; H, 5.67; N, 19.71.

2',3'-O-Isopropylidene derivative (5b) of 5a

A mixture of the isopropylidene derivative of 4a (4b, 98 mg, 0.52 mmol) and 3 (108 mg, 0.62 mmol) in absolute nitromethane (5 ml) containing triethylamine (0.1 ml) was refluxed for 5 h. Triethylamine hydrochloride, precipitated, was filtered off and the filtrate was evaporated in vacuo to give a syrup, which was chromatographed over a silica gel column (1.2 cm x 20 cm) with chloroform-methanol (50 : 1) as an eluent to give 5b (127 mg, 75 % based on 4b) as a yellow foam. MS (m/z): 324

(M^+), 309, 266, 235. Pmr in DMSO-d_6 (δ in ppm): 1.21 and 1.39 (each s, each 3H, 2 x CH_3), 1.51 (m, 1H, H-5'a), 2.15 (m, 1H, H-4'), 2.38 (m, 1H, H-5'b), 3.46 (m, 2H, H-6'), 4.40 - 4.69 (m, 3H, H-1', H-2', and H-3'), 5.00 (bs, 1H, OH), 6.12 (d, 1H, H-5, J = 5.9 Hz), 7.67 (d, 1H, H-6, J = 5.9 Hz), 8.31 (bs, 2H, NH_2), 8.96 (bd, 1H, NH, J = 7.3 Hz).

(±)2',3'-O-Isopropylidene-1-deazaaristeromycin (7)

Compound 5b (290 mg, 0.89 mmol) in ethanol (10 ml) was stirred in the presence of Raney nickel (ca. 50 mg) under a hydrogen atmosphere until the yellow color of the mixture disappeared. The catalyst was filtered off and the solvent was evaporated to give a triamino-derivative (6b) as a colorless syrup. A mixture of 6b and formamidine acetate (151 mg, 1.3 mmol) in methoxyethanol (5 ml) was refluxed for 3 h under a nitrogen atmosphere. The reaction mixture was concentrated in vacuo to give a residue, which was chromatographed over a silica gel column with chloroform-ethanol (10 : 1) as an eluent to afford a crude sample of 7. Recrystallization from water gave 7 (225 mg, 83 % based on 5b), mp 89°(sintered). MS (m/z): 304 (M^+), 289, 274, 259, 215, 134, 133. Pmr in chloroform-d (δ in ppm): 1.38 and 1.60 (each s, each 3H, 2 x CH_3), 2.56 (m, 3H, H-4' and H-5'), 3.85 (m, 2H, H-6'), 4.70 -5.10 (m, 6H, H-1', H-2', H-3', OH, and NH_2), 6.42 (d, 1H, H-1, J = 5.4 Hz), 7.82 (s, 1H, H-8), 7.97 (d, 1H, H-2, J = 5.4 Hz).

Anal. Calcd for $C_{15}H_{20}N_4O_3{\cdot}\frac{1}{2}H_2O$: C, 58.33; H, 6.64; N, 18.14. Found: C, 58.44; H, 6.45; N, 17.90.

(±)1-Deazaaristeromycin (1)[15]

(a) A suspension of 5a (120 mg, 0.42 mmol) in methanol (15 ml) was stirred overnight with Raney nickel (ca. 40 mg) under a hydrogen atmosphere at room temperature. The catalyst was filtered off and the filtrate was concentrated in vacuo to give a crude sample of 6a, which was used in the next step without further purification. A solution of 6a and formamidine acetate (80 mg, 0.45 mmol) in methoxyethanol (5 ml) was refluxed for 2 h. Concentration of the reaction mixture left a residue, which was chromatographed over a Dowex 1 x 2 (OH^- form) column with 30 % aqueous methanol as an eluent to give a crude sample of 1. Recrystallization from water afforded 1 (51 mg. 46 %) in a pure state, mp 214°; $Uv\lambda_{max}^{H_2O}$ (nm): (pH 1), 265sh (ε11,800), 284 (ε16,400); (pH 7) 264

(ε13,300), 278sh (ε11,000); (pH 13) 264 (ε13,800), 278sh (ε10,800). MS (m/z): 264 (M^+), 247, 233, 135, 134. Pmr in DMSO-d_6 (δ in ppm): 1.76 (m, 1H, H-5'a), 2.05 (m, 1H, H-4'), 2.21 (m, 1H, H-5'b), 3.48 (m, 2H, H-6'), 3.84 (m, 1H, H-3'), 4.37 (m, 1H, H-2'), 4.43 - 5.09 (m, 4H, H-1' and OH), 6.33 (m, 3H, NH_2 and H-1), 7.79 (d, 1H, H-2, J = 5.0 Hz), 8.11 (s, 1H, H-8).

Anal. Calcd. for $C_{12}H_{16}N_4O_3$: C, 54.52; H, 6.11; N, 21.20. Found: C, 54.29; H, 5.96; N, 21.12.

(b) A solution of 6b (72 mg, 0.24 mmol) in 50 % formic acid (5 ml) was allowed to stand for 16 h at room temperature. Concentration of the reaction mixture gave a gray residue, which was crystallized from 7 % ammonium hydroxide to yield 1 (51 mg, 80 %). Physical properties of the sample agreed with those of 1 described above.

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$$\log P = 2 - \left(\frac{k \cdot I}{2.303} t \right)$$

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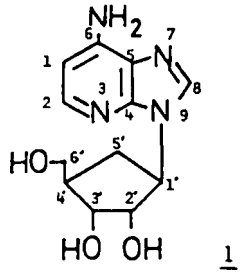

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