SUSCEPTIBILITY TO VARIOUS ENZYMES OF THE CARBON-BRIDGED (*R*) AND (*S*) DIASTEREOISOMERS OF 8,5′-CYCLOADENOSINE AND THEIR 5′-PHOSPHATES

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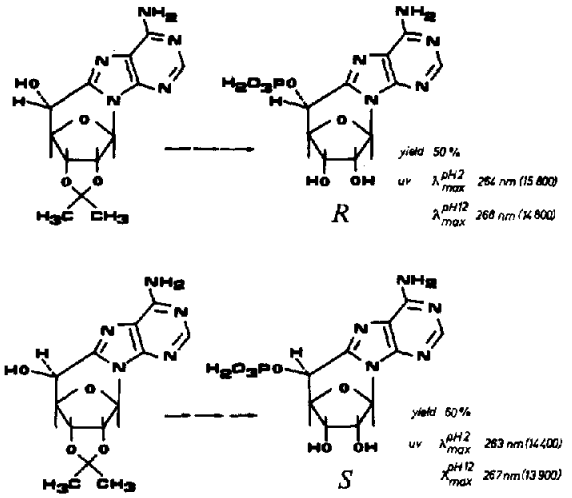
Received 4 September 1979

1. Introduction

The conformational aspects of the interaction of nucleosides and nucleotides with appropriate enzymes has fostered some interest in carbon-bridged cyclonucleosides and their phosphorylated derivatives. Particularly interesting are the purine 8,5′-cyclonucleosides, rigidly fixed in the *anti* conformation. An early synthesis of a mixture of the (*R*) and (*S*) epimers of 8,5′-cycloadenosine [1] and their 5′-phosphates [2] was profited from to examine their substrate properties in several enzyme systems active against adenosine [3] and 5′-AMP [2]. However, the inability to separate the epimers rendered it difficult to ascribe specific substrate properties to each of them. Subsequently, a synthesis of 8,5′-cycloadenosine was described [3,4], including isolation of the two epimers, with results which raised some question as to the validity of the findings in [1,2]. Subsequently, a radiation chemical procedure [5] was applied [6] to convert 5′-AMP selectively to the (*S*) diastereoisomer of 8,5′-cyclo-5′-AMP, identified unequivocally by ^{1}H NMR spectroscopy, and showed that, in contradistinction to the conclusions in [2], this was not a substrate for adenylate kinase or 5′-nucleotidase.

We have now obtained by unambiguous syntheses both the (*R*) and (*S*) epimers of 8,5′-cycloadenosine and the 5′-phosphates of each of them (scheme 1), and have examined their susceptibilities to adenosine deaminase, 5′-AMP deaminase and 5′-nucleotidase.

Scheme 1



The (*R*) and (*S*) epimers of 8,5′-cycloadenosine and their 5′-phosphates

2. Materials and methods

Adenosine, 2′(3′)-AMP and 5′-AMP were products of Pharma-Waldhof (Dusseldorf). *Crotalus adamanteus* 5′-nucleotidase (EC 3.1.3.5), rabbit muscle 5′-AMP deaminase (EC 3.5.4.6) and calf intestinal mucosa adenosine deaminase (EC 3.5.4.4) were obtained from Sigma (St Louis, MO). Enzymatic reactions were carried out as described for 5′-nucleotidase [7], 5′-AMP deaminase [8] and adenosine deaminase [9].

^{1}H NMR spectra of aqueous solutions were recorded on JEOL JNM 4H-100 and Bruker-270 instruments, chemical shifts being measured against internal DSS to an accuracy of 0.01 ppm, and coupling constants to an accuracy of 0.2 Hz. Ultraviolet

absorption spectra were recorded on a Zeiss (Jena) UV–VIS instrument.

3. Results

3.1. *Chemical syntheses*

The (*R*) and (*S*) diastereoisomers of 8,5′-cycloadenosine were synthesized essentially as in [4], via the 2′,3′-*O*-isopropylidene derivatives. Each of the latter was converted to the corresponding 5′-phosphate by the cyanoethylphosphate method [10]. Following removal of the phosphate blocking groups, the isopropylidene derivatives of the nucleotides were purified on Darco-60 charcoal. They were identified by NMR spectroscopy, as well as by their conversion to the starting isopropylidene nucleosides by alkaline phosphatase. The isopropylidene protecting groups were then removed in 0.1 N HCl at 85°C, and the free nucleotides crystallized from water, followed by recrystallization from water, to yield clumps of fine needles for the (*R*) epimer, and long thin rods for the (*S*) epimer. These did not exhibit defined melting points, but decomposed, the (*S*) epimer at 150°C and the (*R*) epimer at 250°C.

3.2. *Identification*

The products were characterized by their NMR and ultraviolet spectra, and by their quantitative conversion by alkaline phosphatase to the parent (*R*) and (*S*) nucleosides, the physico–chemical properties of which were identical with those described [4].

The results of a ^{1}H NMR analysis of the synthesized diastereoisomeric nucleotides fully confirm their structures. The H(8) signal is absent in all of them. The presence of the phosphate group leads to additional coupling between ^{31}P and H(5′), 8.6–8.8 Hz. The 2′,3′-*O*-isopropylidene derivatives exhibit signals due to the methyl groups. $J(H_{4'},H_{5'})$ for the two diastereoisomeric nucleotides are the same as for the parent nucleosides ((*S*) = 6.2 and 5.8 Hz, (*R*) = 0.8 and 0.8 Hz). The values of the vicinal coupling constants all point to a common conformation for the sugar ring of O(1′)*exo* or for neighbouring conformations in the pseudorotational model of the ribofuranose ring [11], i.e., O(1′)*exo*–C(1′)*endo* and O(1′)*exo*–C(4′)*endo*. This characteristic conformation for the diastereoisomeric nucleosides and nucleotides is clearly dictated by the C(8)–C(5′) linkage and is unaltered on introduction of the 2′,3′-*O*-isopropylidene group.

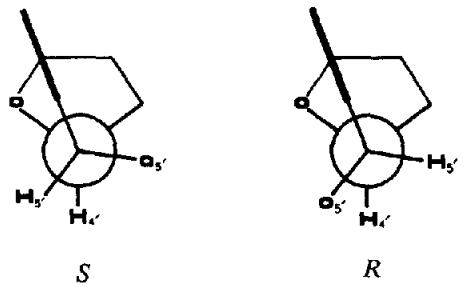
Identification of each epimer was based on the agreement between the coupling constants of H(4′) and H(5′) from the NMR spectrum and those predicted by the Karplus relation [12] for the corresponding torsion angles between H(4′) and H(5′) from molecular models and Newman projections (scheme 2) for the 8,5′-cyclonucleosides. A coupling constant of 6 Hz corresponds to an angle of ~40°, and 0.8 Hz to ~80°. Our spectrum for the (*S*)-epimer coincides with that reported [6].

3.3. *Enzymatic tests*

Both diastereoisomers of 8,5′-cycloadenosine were fully resistant to adenosine deaminase; the epimer (*S*), but not (*R*), was only a weak inhibitor of adenosine deaminase, with a $K_i = 3 \times 10^{-4}$ M. The 5′-phosphates were also fully resistant to 5′-AMP deaminase; under conditions where 5′-AMP was completely deaminated in 1 min, no detectable deamination of either of the isomers was noted after 60 min incubation.

Under conditions where 2′(3′)-AMP is unaffected, and 5′-AMP is fully dephosphorylated by 5′-nucleotidase in 3–4 min, 8,5′(*S*)-cycloadenosine-5′-phosphate is unaffected after 18 h incubation, while the (*R*) diastereoisomer is slowly (at ~0.5% of the rate for 5′-AMP) hydrolyzed quantitatively to the nucleoside. Both the diastereoisomeric nucleotides, and their 2′,3′-*O*-isopropylidene derivatives, were readily and quantitatively converted to the corresponding nucleosides by alkaline monophosphoesterase.

Scheme 2



S *R*

Newman projections for the C(5′) epimers of 8,5′-cycloadenosine and its 5′-phosphate

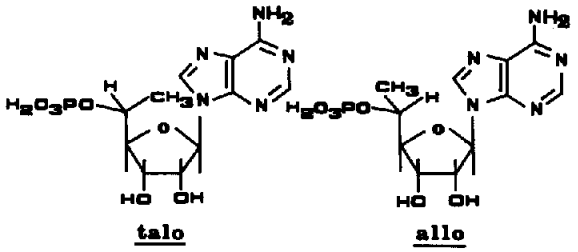
4. Discussion

The present results are in contradiction with the report [2,3] that: (a) one of the diastereoisomeric nucleosides is deaminated by adenosine deaminase at 0.1, the other at 0.01, the rate for adenosine; (b) both nucleotide isomers were substrates of 5′-AMP deaminase; (c) one of the nucleotide isomers, the (*S*) epimer, is as effective a substrate for 5′-nucleotidase as 5′-AMP. Our findings are in agreement with the observation [6] that 8,5′-(*S*)-cycloadenosine-5′-phosphate is not a substrate for 5′-nucleotidase.

It should, furthermore, be noted that formation of the 8,5′-linkage profoundly modifies the nature of the exocyclic group. Both epimeric nucleotides possess, not a primary 5′-hydroxyl as in 5′-AMP, but a secondary hydroxyl. This in itself, even with a favourable spatial orientation of substrate relative to enzyme, would be expected to markedly decrease the rate of hydrolysis by 5′-nucleotidase. And, in fact, the (*R*) nucleotide is dephosphorylated 200–300-times slower than 5′-AMP; the specificity of even this slow rate is testified to by the resistance to dephosphorylation, under these conditions, of 2′(3′)-AMP, in which the phosphorylated hydroxyls are also secondary hydroxyls.

It consequently becomes of interest to compare the substrate properties of the (*R*) and (*S*) AMP analogues with those of analogues containing a non-carbon-bridged secondary 5′-hydroxyl esterified with phosphoric acid. Such analogues are known, viz., *talo*-5′-AMP and *allo*-5′-AMP (scheme 3). Like our

Scheme 3



Talo-5′-methyl-AMP and *allo*-5′-methyl-AMP

8,5′-(*R*)-cycloadenosine-5′-phosphate, they are feeble substrates for 5′-nucleotidase, and are hydrolyzed 200–400-times slower than 5′-AMP [13]. From these data the conformation of 5′-AMP which interacts with 5′-nucleotidase may be defined as *anti,gauche–trans*. Consistent with this is the demonstration that model purine nucleoside-5′-phosphates in the conformation *syn* are very feeble, or inactive as, substrates for 5′-nucleotidase [14].

Acknowledgements

We are indebted to Ryszard Stolarski for assistance in interpretation of the NMR spectra. This investigation was supported by the Polish National Cancer Research Program (PR-6/1701).

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