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IDENTIFICATION OF THE BIOACTIVE ENANTIOMER OF *ERYTHRO*-3-(ADENIN-9-YL)-2-NONANOL (EHNA), A SEMI-TIGHT BINDING INHIBITOR OF ADENOSINE DEAMINASE*

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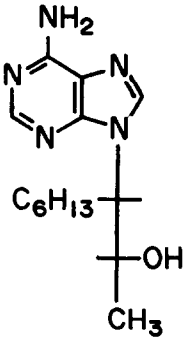
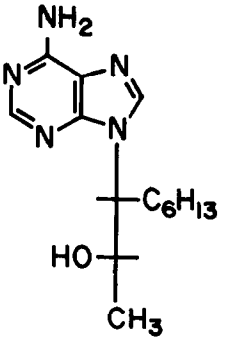
In the past few years, inhibitors of adenosine deaminase (ADA, adenosine aminohydrolase EC 3.5.4.4) have figured importantly as potential co-drugs for use with certain adenine nucleosides that are active antiviral and/or antitumor agents. Of these, pentostatin [1, 2] and coformycin [3] are extremely potent, tight-binding inhibitors of the enzyme having K_i = 2.5 x 10^{-12} and 1.0 x 10^{-10} M, respectively, against human erythrocytic ADA [4]. EHNA ["*erythro*-9-(2-hydroxy-3-nonyl)adenine," *erythro*-3-(adenin-9-yl)-2-nonanol], on the other hand, is classified as a semi-tight binding inhibitor, with a K_i = 1.6 x 10^{-9} M [4], and was the first of the ADA inhibitors shown [5] to potentiate the antitumor effects of adenine nucleosides such as cordycepin and 9-β-D-arabinofuranosyladenine (ara-A). Owing to its reversible nature and potentially lower toxicity [6], EHNA is advocated as possibly the choice of inhibitor for use with antiviral agents such as ara-A [7].

EHNA is a synthetic product, the result of extensive studies by Schaeffer and co-workers to design precisely the ideal molecule that would effectively bridge to the hydrophobic, the hydrophilic and the methyl-binding regions of the enzyme [8]. While the *erythro* diastereomers were shown to be stronger inhibitors than their *threo* counterparts, it has generally been assumed and has recently been demonstrated [9] that only one of the *erythro* enantiomers was responsible for the enzymic activity. To date no attempts toward identifying the bioactive enantiomer have been reported. To this end we have synthesized in an unambiguous manner from carbohydrate precursors both D- and L-EHNA and have evaluated each stereoisomer separately against calf mucosal ADA (Sigma Type I, pH 7.5 phosphate buffer, 265 nm). Using the pre-incubation technique developed for compounds of these types [4], we found that the L-isomer **2** was bound some 80-fold more tightly than the D-isomer **1**. Lineweaver-Burk plots, obtained using steady-state rates established after a 4-5 min incubation, for **1** and **2** are depicted in Fig. 1.



D-EHNA (1)



L-EHNA (2)

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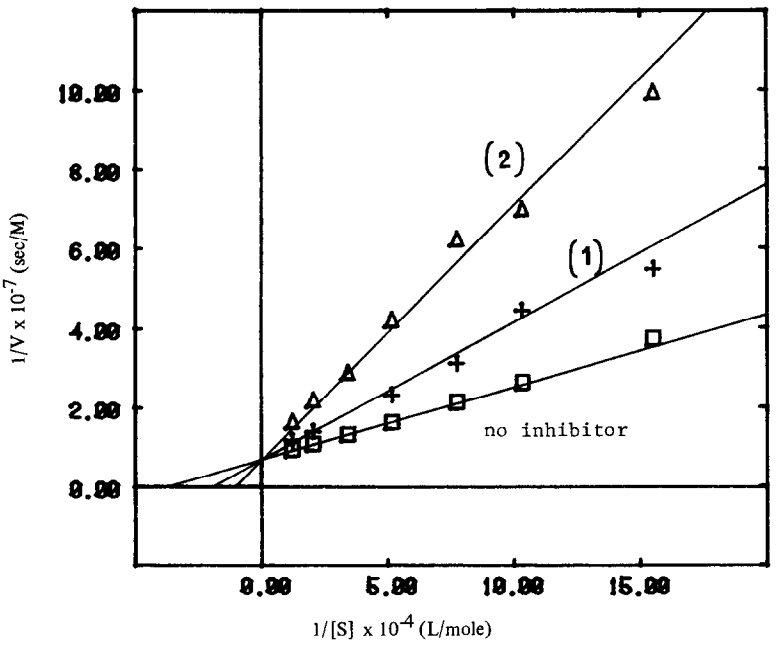

Fig. 1. Competitive inhibition of adenosine deaminase by **1** and **2** (adenosine as substrate). [1] 2.61 x 10^{-8} M and [2] 1.91 x 10^{-9} M. The K_i values were established as follows: D, L-EHNA*, 1.38 x 10^{-9} M; D-EHNA† (1), 6.23 x 10^{-8} M; L-EHNA† (2), 7.64 x 10^{-10} M.

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* Kindly supplied as EHNA·HCl by Burroughs-Wellcome Co., Research Triangle Park, NC 27709.

† Details of the syntheses will be published elsewhere. Beginning with 1,2:3,4-di-*O*-isopropylidene-D- or L-rhamnitol (**3a** or **3b**), the D- and L-EHNA's were synthesized as follows: **3a** or **3b** was converted to the 5-*O*-benzyl derivative **4a** or **4b**, with benzyl chloride–sodium hydride in DMF; acid hydrolysis of **4a** or **4b**, followed by periodate cleavage, gave the (*R*) - or (*S*) - 2-benzyloxypropanal (**5a** or **5b**); reaction of **5a** or **5b** with hexylmagnesium bromide furnished a 25:75 mixture of 2(*R*), 3(*S*)-2-*O*-benzyl-2,3-nonanediol (**6a**) (or the 2(*S*), 3(*R*)-isomer, **6b**) and 2(*R*), 3(*R*)-2-*O*-benzyl-2,3-nonanediol (**7a**) (or the 2(*S*), 3(*S*)-isomer, **7b**); **7a** or **7b** was converted to the respective methanesulfonyl derivative and subsequently reacted with the sodium salt adenine to give 2(*R*), 3(*S*)-2-*O*-benzyl-3-(adenin-9-yl)-2-nonanol (**8a**) (or the 2(*S*), 3(*R*)-isomer, **8b**); **8a** or **8b** was hydrogenolyzed to give each *erythro* product, D-EHNA (**1**) and L-EHNA (**2**). Physical data for **1**: (m.p. = 206-207°; $[\alpha]_D^{20}$ = -31.7° (0.5% w/v, ethanol); physical data for **2**: (m.p. = 205-207°; $[\alpha]_D^{20}$ = +30.1° (0.5% w/v, ethanol). Both **1** and **2** were found to be identical with authentic EHNA·HCL, by m.p., T.L.C., I.R., N.M.R. and elemental analysis.

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