A Novel Adenosine and Ara-A Deaminase Inhibitor, (R)-3-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-3,6,7,8-tetrahydroimidazo[4,5-*d*][1,3]diazepin-8-ol

Peter W. K. Woo and Henry W. Dion

Chemistry Department, Research and Development Division, Parke, Davis and Company, Ann Arbor, Michigan 48106

Siegfried M. Lange and Lawrence F. Dahl

Department of Chemistry, University of Wisconsin, Madison, Wisconsin 53706

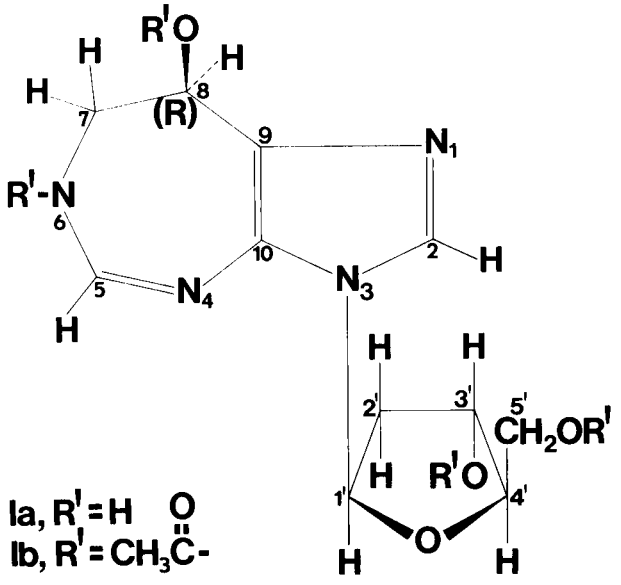
Lois J. Durham

Department of Chemistry, Stanford University, Stanford, California 94305

Received June 21, 1974

Sir:

From the fermentation broth of a strain of *Streptomyces antibioticus*, a potent adenosine and ara-A deaminase inhibitor (compound Ia), $C_{11}H_{16}N_4O_4$, was isolated in crystalline form, m.p. 220-225°, $[\alpha]_D^{25}$ +76.4° (c 1, water), pK_a' 5.2 in water (1). We wish to present the structural elucidation of Ia, with comments on its possible mode of action and relationships with certain other deaminase inhibitors.



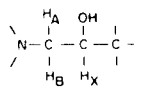
The composition of Ia was established by elemental analyses and high resolution mass spectrometry (molecular ion m/e 268.11771). Compound Ia shows uv λ max 282 (ε 8000) in water at *p*H 7, λ max 283 (ε 7970) at *p*H 11, and λ max 273 (ε about 7570 initially, decreasing to 3143 after 6.5 hours) at *p*H 2. The decrease of ε value at *p*H 2 is accompanied by the loss of deaminase inhibitory activity, indicating the lability of the chromophore in acid. Hydrolysis of Ia in 0.02 *N* aqueous acetic acid heated under reflux for two hours yielded 2-deoxy-D-*erythro*-pentose (2-deoxy-D-ribose), characterized as the crystalline *N*-phenylglycosylamine (2). Acetylation of Ia in acetic anhydride and pyridine yielded an amorphous tetraacetate (Ib), m.p. 68-78°, $C_{11}H_{16}N_4O_4 \cdot (C_2H_2O)_4$ (established by elemental analyses and ms molecular ion peak at m/e 436).

The existence of the deoxypentose moiety as an *N*- or *O*-glycoside is indicated by the low-field position of the nmr signals for the anomeric proton H-1′ (δ 6.58-6.72, $J_{1',2''}$'s = 6.3, 7.8 Hz in Ia; δ 6.76-6.89, $J_{1',2''}$'s = 6.8, 6.8 in Ib) (3a,b) and by ms peaks which correspond to the "sugar" fragment and the aglycone "base +1" fragment resulting from the cleavage of the C-1′-N bond (4) (m/e 117 and 152 from Ia; 201 and 236 from Ib). The existence of the deoxypentose moiety in the furanose rather than pyranose form is indicated by a comparison of the nmr signal positions of protons H-3′, H-4′, and H-5′ in Ia relative to those in Ib (H-3′, δ 4.90-5.03 in Ia, 5.80-5.97 in Ib;

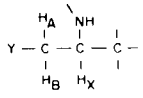
H-4′, δ 4.46-4.57 in Ia, *ca.* 4.83 in Ib; H-5′, δ 4.00 to 4.31 to Ia, *ca.* 5.73 in Ib). The large downfield shifts observed for H-3′ (*ca.* 0.92 ppm) and H-5′ (*ca.* 1.57 ppm) show that the hydroxyl groups at C-3′ and C-5′ are free in Ia and acetylated in Ib, and the small shifts for H-4′ (*ca.* 0.32 ppm) shows that the oxygen at C-4′ is not affected by acetylation of Ia and consequently must exist as part of a furanose ring.

As may be deduced from data presented above, the aglycone must have a composition of $C_6H_7N_4O$, with two acylable groups (-OH or NH). Additional structural information is deduced as follows.

The ^1H nmr spectra of compounds Ia and Ib indicate the presence of an ABX type of proton grouping such as a-d (3a, b).



a



b Y = -O-

c Y = -C=O

d Y = 

In the spectrum of Ia, H_X appears as a quartet at δ 5.51-5.57, H_A and H_B as two sets of quartets at δ 3.65-4.03, J_{AB} = 13.6 Hz, J_{AX} and J_{BX}, 1.8 and 4.3 Hz (the 1.8-Hz splittings occurring at higher field). In the spectrum of Ib, H_A appears as two slightly boradened peaks at δ 3.58-3.73, H_B as a quartet at δ 5.13-5.33, H_X as a slightly broadened doublet at δ 6.55-6.59, J_{AB} = 15.0 Hz, $J_{AX} \leq 1$ Hz, J_{BX} = 3.5 Hz. (In groupings b-d, the low field position of H_X compared to those of H_A and H_B, while somewhat anomalous, is not impossible; reported examples incorporating grouping b are represented by the two C-6 isomers of VId (5).

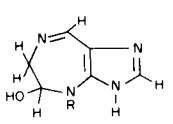
The nmr spectrum of Ia shows two aromatic proton singlets at δ 7.60 and 8.08 (3a). In dimethyl sulfoxide-d_6, the singlet at higher field is split into a doublet, J 4.7 Hz. The splitting is evidently caused by a proton exchangeable with deuterium oxide, and grouping e or f is therefore indicated.

e

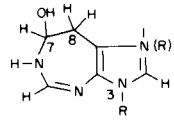
f

The ^{13}C nmr spectrum of compound Ia (6) shows signals corresponding to 11 carbon atoms. Peaks originating from the five deoxypentosyl carbon atoms may be assigned on the basis of ^{13}C-H splitting patterns and from assignments reported for 2-deoxyadenosine (III) and α(and β)-2-deoxy-*erythro*-pentofuranose (IV) (7): C-1′, 84.3 ppm (d) [84.8 in III, 91.65 in IV (α), 98.05 in IV (β)]; C-2′, 39.9 (t) (39.15 in III; 41.15, 41.3 in IV); C-3′, 72.1 (d) (70.9 in III; 70.95, 71.25 in IV); C-4′, 87.7 (d) (87.5 in III; 85.3, 85.85 in IV); C-5′, 62.6 (t) (61.4 in III; 61.55, 62.85 in IV). The remaining peaks due to the aglycone carbon atoms are assigned (8) as follows: 150.8 ppm (d) and 132.3 (d), two of $\rangle$C=; 130.6 (s) and 129.5 (s), two of >C=; 67.6 (d), >CH-O- or >CH-N<; 47.9 (t), $-CH_2-N<$ or $-CH_2O-$, somewhat outside the range of $-CH_2-$ (23 to 45 ppm).

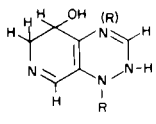
The data presented and structural groupings deduced above are entirely consistent with structure Ia but may also accommodate structures such as Va to Ve (R = 2-deoxy-D-*erythro*-pentofuranosyl) and VIa.



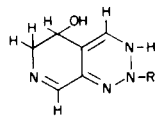
Va



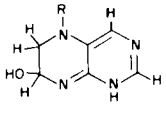
Vb



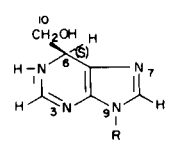
Vc



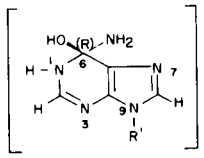
Vd



Ve



VIa, R = 2-deoxy-D-*erythro*-pentofuranosyl
b, R = β-D-ribofuranosyl; C-6 configuration unknown
c, R = β-D-ribofuranosyl; C-6 (S) configuration
d, R = H



VIIIa, R′ = 2-deoxy-D-*erythro*-pentofuranosyl
b, R′ = β-D-ribofuranosyl; C-6 configuration unknown
c, R′ = β-D-ribofuranosyl; C-6 (R) configuration
d, R′ = β-D-arabinofuranosyl; C-6 (R)

As an inhibitor of the enzymatic deamination of adenosine (VIIa) or ara-A (VIIb) (1b), compound Ia may be expected to have a structure closely resembling the substrates VIIa and VIIb (9) or the reactive intermediate VIIIb proposed for the enzymatic deamination of VIIa (*cf.* below) (10). Among the structural possibilities, structure Ia, Vb, and VIa, with 2-deoxy-β-D-*erythro*-pentofuranosyl moiety at N-3, N-3, and N-9, respectively, should decidedly bear the closest resemblance to VIIa, VIIb, and VIIIb. Isomers of Ia, Vb, and VIa, with the deoxpentosyl moiety at N-1, N-1, and N-7, respectively, or in the α-anomeric configuration, bear much less resemblance. Furthermore, structure Vb is less favored by ^{13}C nmr data (C-8 expected to be at higher field than 47.9 ppm; C-7 expected to be at lower field than 67.6 ppm) (8). Structure VIa is not favored by uv data, since the two C-6 isomers of VIb (one of which is a ribosyl analog of VIa) show uv λ max at 291 and 293 mμ, respectively (10b), rather than 283 mμ as shown by compound Ia.

Thus, from consideration of chemical and physical data and the additional assumed requirement of structural resemblance to the substrate of enzymatic catalysis, structure Ia (C-8 configuration unspecified) emerges as the most likely of several possible structures.

The structure of compound Ia was finally determined in a conclusive way by single crystal X-ray diffraction techniques (11). The molecule crystallized from methanol in the monoclinic space group $P2_1$, with unit cell constants a = 11.318 (3), b = 10.774 (3), c = 4.995 (1) Å, β = 101.55 (2)°. The structure was found by direct methods using weighted tangent formula refinement (12), and all hydrogen atoms were located in a difference electron synthesis. Refinement by full-matrix least squares was based on 841 independent diffractometer data. At the end of the process, isotopic parameters for all hydrogen atoms were allowed to vary. Convergence was attained at final discrepancy indices of R_1 = 0.042 and R_2 = 0.054. The structure as represented by Ia was thus established.

"Transition-state analogs," *i.e.*, stable molecules with enzyme-binding properties resembling those of highly reactive substrate intermediates approaching the transition state, are expected to be unusually potent enzyme inhibitors (10). Thus, the activity of one of the two C-6 stereoisomers of VIb, a potent adenosine deaminase inhibitor, was explained by its being an analog of the substrate intermediate VIIIb (10). The deaminase inhibitory activity of Ia may be explained on a similar basis. A significant common feature among Ia, VIb, and VIIIb is the tetrahedral carbon at C-8, C-6, and C-6, respectively.

Space-filling models of Ia, VIc, and VIIIc (or VIIId, most likely the corresponding intermediate in the enzymatic deamination of ara-A), possessing 8(R), 6(S), and 6(R) configurations (13), respectively, show that, relative to the imidazole ring and the nitrogen attached to it (*i.e.*, N-3 of VIc and VIIIc, N-4 of Ia), the position of the C-6 hydroxyl of VIIIc can resemble closely that of the C-8 hydroxyl of Ia, and, less closely, that of the C-10 hydroxyl of VIc, while the positions of the C-6 amino of VIIIc, the C-8 hydrogen of Ia, and the C-6 hydrogen of VIc are almost identical. Preliminary results showed that Ia was probably more than 100 times as potent in deaminase inhibitory activity as VIc. These tentative configurational assignments, if correct, would tend to support the possibility of an enzyme-inhibitor complex in which an oxygen from the inhibitor is binding the enzyme site normally occupied by the C-6 oxygen of VIIIc (or VIIId), which has originated from enzyme-bound water, while a hydrogen is taking the site normally occupied by the amino leaving group (*cf.* ref 9 and 10).

According to properties reported, coformycin (14), another potent adenosine deaminase inhibitor, is most likely a D-ribofuranosyl analog of Ia; *i.e.*, the aglycone is most likely the same in both compounds.

Acknowledgements.

The authors acknowledge their appreciation to Prof. Harry S. Mosher and Dr. Malcomb Bramwell, Stanford University, for helpful discussions and assistance.

REFERENCES

(1a) U. S. Patent applied for. (b) Ara-A is the abbreviation of 9-(β-D-arabinofuranosyl)adenine, J. D. Howells and A. Ryder (Parke, Davis and Company), U. S. Patent 3,616,208, 26 October, 1971.

(2) L. Hough, *J. Chem. Soc.*, 3066 (1953).

(3a) Spectra were determined in deuterium oxide at 100 MHz with chemical shifts reported in ppm from external tetramethylsilane. (b) Signal assignments were confirmed by spin-decoupling studies at 100 MHz.

(4) K. Biemann and J. A. McCloskey, *J. Am. Chem. Soc.*, **84**, 2005 (1962).

(5a) J. S. Connolly, Ph.D. Thesis, Brandeis University, 1969. (b) H. Linschitz and J. S. Connolly, *J. Am. Chem. Soc.*, **90**, 2979 (1968).

(6a) Spectra were determined in deuterium oxide using dioxane (δ_C 67.4 ppm from tetramethylsilane) as internal reference. (b) Multiplicities of ^{13}C-H splittings were determined with off-resonance proton decoupling.

(7) E. Breitmaier, G. Jung, and W. Voelter, *Chimia*, **26**, 136 (1972).

(8) E. Breitmaier, G. Jung, and W. Voelter, *Angew. Chem. Intern. Edit. Engl.*, **10**, 673 (1971).

(9) J. G. Cory and R. J. Suhadolnik, *Biochemistry*, **4**, 1729 (1965).

(10a) R. Wolfenden, *Accounts Chem. Res.*, 5, 10 (1972). (b) B. Evans and R. Wolfenden, *J. Am. Chem. Soc.*, **92**, 4752 (1970).

(11) S. M. Lange, L. F. Dahl, and P. W. K. Woo, to be submitted for publication in *J. Am. Chem. Soc.*

(12) G. Germain, P. Main, and M. M. Woolfson, *Acta Cryst.* A 27, 368 (1971).

(13) R. S. Kahn, C. K. Ingold, and V. Prelog, *Experientia*, **12**, 81 (1956).

(14a) T. Tsuruoka, N. Ezaki, S. Amano, C. Uchida, and T. Niida, *Meiji Seika Kenkyu Nempo (Yakuhin Bumon)*, 17 (1967). (b) T. Sawa, Y. Fukagawa, I. Homma, T. Takeuchi, and H. Umezawa, *J. Antibiot.* (Tokyo), Ser. A, **20**, 227 (1967).