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The 6-Azauracil and 6-Azathymine Nucleosides

of 2-Deoxy- D -arabinohexopyranose (1)

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The current interest in the specific inhibition of the enzyme, pyrimidine nucleoside phosphorylase (3,4), by 1-(2-deoxy-β-D-arabinohexopyranosyl)-thymine ("2-deoxyglucosylthymine") (5) has prompted us to prepare two new azapyrimidine nucleosides containing the 2-deoxy-D-glucose moiety, namely, 1-(2-deoxy-D-arabinohexopyranosyl)-6-azauracil [2-(2-deoxy-D-arabinohexopyranosyl)-as-triazine-3,5(2H,4H)-dione] (Va) and 1-(2-deoxy-D-arabino-hexopyranosyl)-6-azathymine [2-(2-deoxy-D-arabino-hexopyranosyl)-6-methyl-as-triazine-3,5(2H,4H)-dione] (Vb). Recently, additional 2-deoxyhexosyl nucleosides have been synthesized and screened in this enzyme system (6,7). As a result of this work it has been observed that in terms of the carbo-hydrate moiety the structural requirements for inhibition of this enzyme appear to be highly specific (7). It is hoped that the azapyrimidine nucleosides described in this paper will indicate to some extent the pyrimidine requirements for the inhibition of this enzyme by 2-deoxy-D-glucosyl pyrimidine nucleosides. In addition, since the anticancer activity of 6-azauridine is well known (8), these nucleosides may be considered as potential anticancer "fraudulent" nucleosides.

The direct synthesis of the nucleosides of 6-aza-uracil and 6-azathymine is complicated in the case of the mercury-method by the formation of a mixture of N-1 and N-3 isomers (9), and is not applicable in the case of the Hilbert-Johnson method because the necessary 3,5-dialkoxy-as-triazines are not known (10). The application of the method of Nishimura and Iwai (11) which uses 2,4-bis-(trimethylsilyloxy)-pyrimidines in a modified Hilbert-Johnson reaction has been shown to yield the desired N-1 nucleosides in the case of the 5-bromo- and 5-trifluoromethyl-6-azauracils and 6-azauracil itself (12a-c).

The required 3,5-bis-(trimethylsilyloxy)-as-tri-azine (IIa) and 3,5-bis-(trimethylsilyloxy)-6-methyl-as-triazine (IIb) were prepared from 6-azauracil (Ia) (13) and 6-azathymine (Ib) (13), respectively, by re-action with hexamethyldisilazane (14). The coupling of IIa and IIb with 2-deoxy-3,4,6-tri-O-p-nitro-benzoyl-α-D-arabinohexosyl bromide (III) (15) was carried out according to the method of Hilbert and Johnson (16). The resulting protected crystalline nucleosides (IVa, IVb) were deacylated by methoxide catalyzed transesterification to yield the desired nucleosides (Va, Vb).

The β-configuration is tentatively assigned for these nucleosides (Va, Vb) on the basis of their low specific rotations, (Va, $[\alpha]_D^{24°} = +0.1°$; Vb, $[\alpha]_D^{28°} = -1.4°$), since the specific rotations of the pure 2-deoxy-β-D-glucopyranosyl nucleosides of uracil (7) and thymine (3,5) are +5.6° and +4.0° (+4.6°), and the specific rotations of their anomeric pairs (3) are +85.5° and 89.6°. The patterns of the anomeric protons of these nucleosides, Va and Vb, appear in the n.m.r. spectra as a doublet of
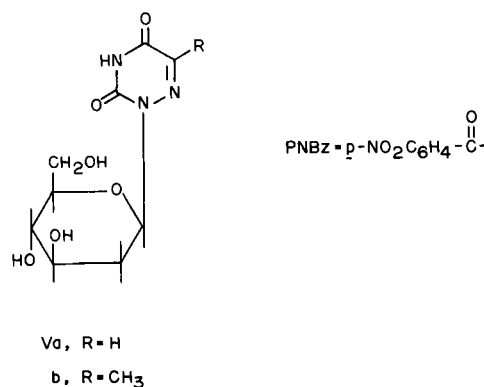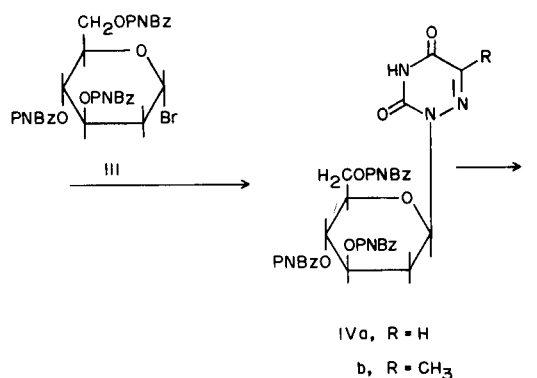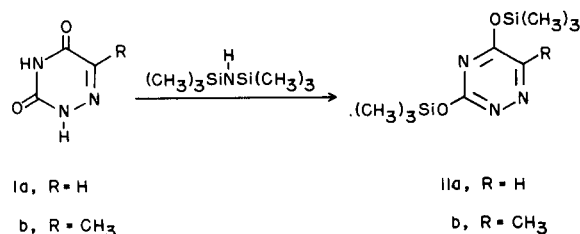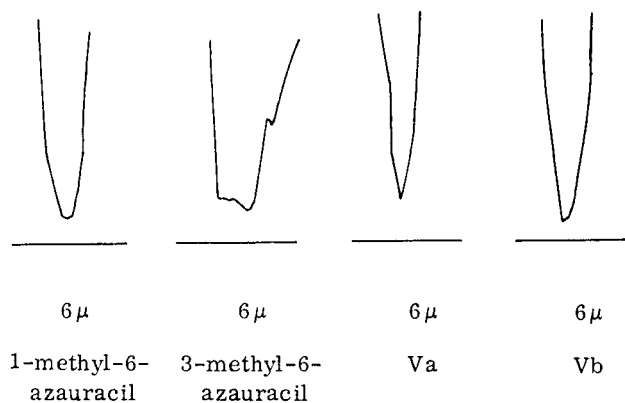

Ia, R = H

b, R = CH$_3$

IIa, R = H

b, R = CH$_3$

III

IVa, R = H

b, R = CH$_3$

PNBz = p-NO$_2$C$_6$H$_4$-C-

Va, R = H

b, R = CH$_3$

TABLE I

Carbonyl Stretching Bands in Nujol

6 μ	6 μ	6 μ	6 μ
1-methyl-6-azauracil	3-methyl-6-azauracil	Va	Vb

doublets centered at τ, 4.2 $J_{axial-axial}$ 9 cps and $J_{axial-eq}$. 3-4 cps. Since the β-anomer has axial-axial, C'_1-C'_2, protons and axial-axial coupling constants are of the order of 6-10 cps this indicates the β-configuration. Whereas, the α-anomer would not have axial-axial, C'_1-C'_2, protons and thus would have a much smaller coupling constant. The spectra verified the presence of only one anomer. A Varian A-60 n.m.r. spectrometer was used. Proton chemical shifts (ppm τ) were measured from tetramethylsilane as internal reference in a mixture of acetone$_6$/dimethylsulfoxide$_6$.

The assignment of the carbohydrate moiety to the N-1 position of the 6-azauracil and 6-azathymine rings of these nucleosides is based on the fact that they exhibit a hypochromic shift of the absorption maximum in the ultraviolet spectra on the change from acidic to basic conditions. This is typical of N-1 substituted 6-azauracils (17). In addition, the infrared absorption in the carbonyl stretching region confirms the N-1 substitution of the 6-azauracil component since the spectra of these nucleosides are similar to that of 1-methyl-6-azauracil and different from 3-methyl-6-azauracil (Table I). The use of the infrared spectra to distinguish between N-1 and N-3 substituted 6-azauracils and nucleosides is described by M. Horak and J. Gut (18).

The difference between the ultraviolet spectra of these compounds, e.g. Va, λ max (pH 7 buffer), 254 mμ, and 1-(2-deoxy-β-D-ribofuranosyl)-6-azauracil, λ max (EtOH), 265 mμ (12c), is partially due to a solvent effect since the λ max (95% EtOH), of Va and Vb are 261 mμ and 263 mμ respectively.

EXPERIMENTAL (19)

3,5-Bis-(trimethylsilyloxy)-as-triazine (IIa) (20).

A mixture of 3.9 g. (0.034 mole) of 6-azauracil (Ia) (13) and 30 ml. of hexamethyldisilazane was refluxed for 2 hours at which time a clear solution was observed and the temperature had risen to 141°.
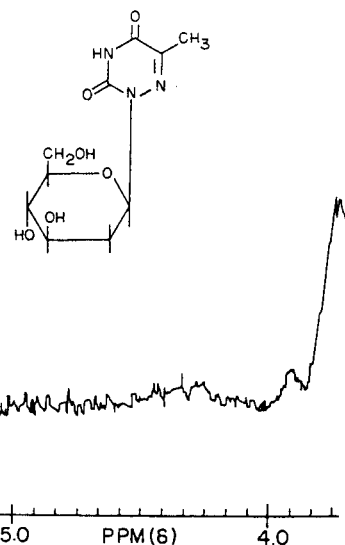
The excess hexamethyldisilazane was removed under vacuum and the residue distilled, b.p. 142-144° at 25 mm. giving 7.9 g. (90%) of the product (IIa). On standing IIa solidified, m.p. ca. 35°. Due to the high reactivity of this compound, the attempted analysis resulted only in a percent composition corresponding to that of 6-azauracil. The infrared spectra showed a strong band at 9.32 μ (film), indicating the presence of the [(CH₃)₃SiO-] group (11).

1-(2-Deoxy-3,4,6-tri-O-p-nitrobenzoyl-D-arabinohexosyl)-6-azauracil (IVa).

To 5.1 g. (20 mmoles) of 3,5-bis-(trimethylsilyloxy)-as-triazine (IIa) was added 3.5 g. (5.2 mmoles) of 2-deoxy-3,4,6-tri-O-p-nitrobenzoyl-α-D-arabinohexosyl bromide (III) (15). The mixture was stirred and heated at 100° under vacuum for 25 hours, at which time it had solidified. The solid mixture was extracted three times with 250-ml. portions of chloroform containing 2 ml. of absolute ethanol. The ethanol was added to remove the trimethylsilyl groups from the triazine ring. The chloroform extracts were evaporated in vacuo at 60° to an oil. This oil on digestion with 200 ml. of boiling absolute ethanol slowly solidified, and the solid crude protected nucleoside (IVa) (2.1 g., m.p. 235-250°) was removed by filtration of the hot mixture. This crude material was recrystallized from glacial acetic acid containing a trace of water, yielding 720 mg. of product, m.p. 241-242°, recrystallizing and melting at 287.5-288.5°. An additional 450 mg. (m.p. 235-237° recrystallizing and melting at 286-288°) was obtained from the mother liquors, giving a yield of 32%. In one instance, this compound was obtained as an interconvertible polymorphous form (m.p. 250-252°) from glacial acetic acid.

Anal. Calcd. for $C_{30}H_{22}N_6O_{15}$: C, 51.00; H, 3,14; N, 11.90. Found: C, 51.02; H, 3.13; N, 11.74.

1-(2-Deoxy-D-arabinohexopyranosyl)-6-azauracil (Va).

A mixture of 1.17 g. (1.66 mmoles) of pure protected nucleoside (IVa) and 40 ml. of absolute methanol was treated with 4 ml. of 1 N (4.0 mmoles) sodium methoxide in absolute methanol. The resulting clear solution (pH 11) was stirred over night at room temperature and then evaporated to an oil at 60° in vacuo. This oil was treated with 25 ml. of water, made acidic (pH 1) with concentrated hydrochloric acid, and washed twice with 25-ml. portions of chloroform. The aqueous layer was then concentrated to an oil in vacuo. The residue was extracted with 100 ml. of hot acetone, filtered, and the filtrate concentrated in vacuo to 10 ml. On the addition of 10 ml. of ethyl acetate, 130 mg. of Va was obtained. An additional 210 mg. of product was recovered from the mother liquors, giving a 79% yield of Va, m.p. 214-215.5° (21), $[\alpha]_D^{24}$ +0.1° (c 0.50 water), λ max (0.1 N HCl), 259 mμ (log ϵ 3.74), λ min (0.1 N HCl), 227 mμ, λ max (pH 7 buffer), 254 mμ (log ϵ 3.82), λ min (pH 7 buffer), 220 mμ, λ max (0.1 N NaOH), 252 mμ (log ϵ 3.79), λ max (Nujol), 5.95 μ (C=O).

Anal. Calcd. for $C_9H_{13}N_3O_6$: C, 41.70; H, 5.05; N, 16.21. Found: C, 41.86; H, 5.12; N, 16.30.

3,5-Bis-(trimethylsilyloxy)-6-methyl-as-triazine (IIb).

A mixture of 5.08 g. (0.04 mole) of 6-azathymine (Ib) (13) and 20 ml. of hexamethyldisilazane was refluxed for 2 hours. During this
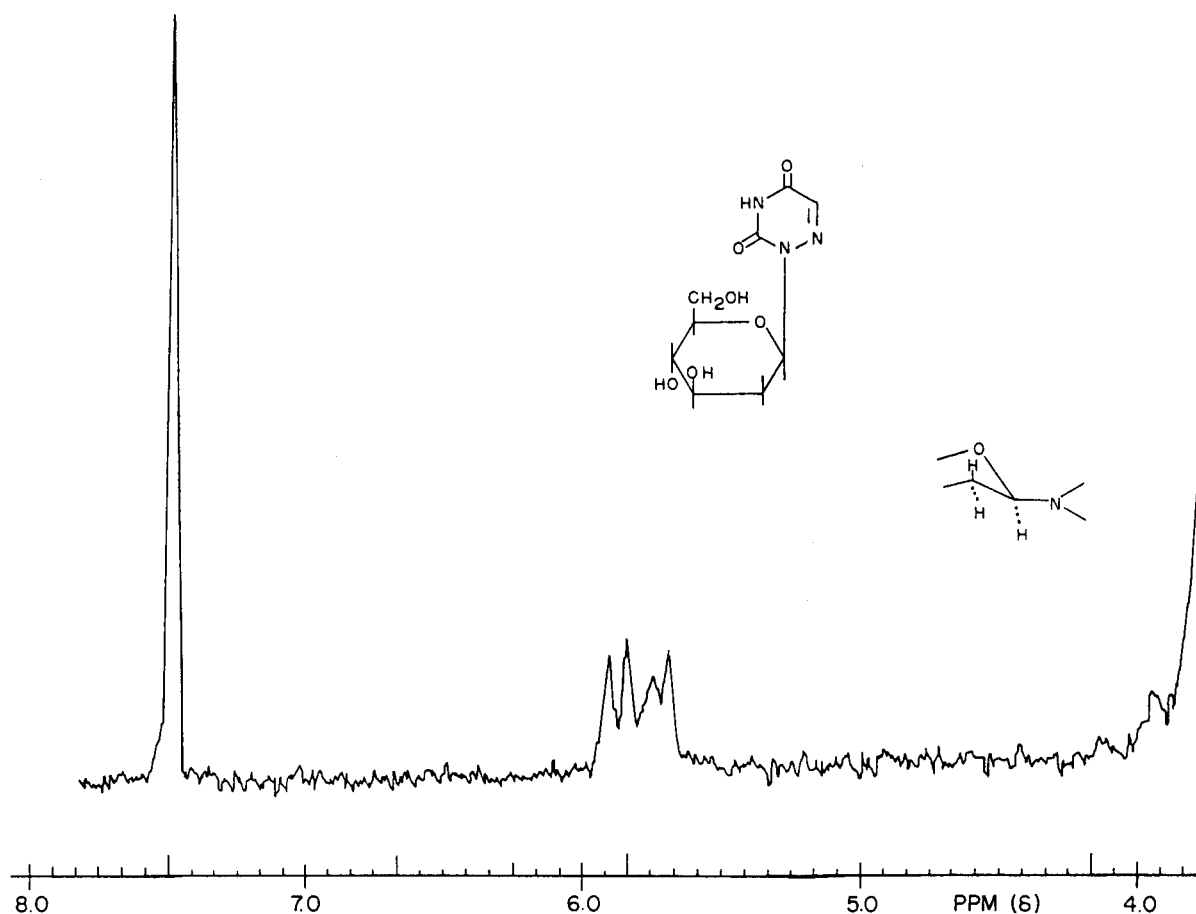
time, the mixture became clear and the temperature rose from 115 to 140°. The excess solvent was removed under vacuum at 20 mm. and the product distilled, b.p. 146-147° at 20 mm., giving 10.35 g. (95%) of IIb, λ max (film), 9.46 μ [(CH₃)₃SiO-]. Due to the high reactivity of this intermediate, analysis was not attempted.
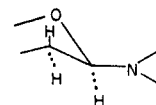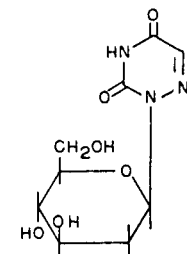
1-(2-Deoxy-3,4,6-tri-O-p-nitrobenzoyl-D-arabinohexosyl)-6-azathymine (IVb).

A mixture of 6.9 g. (22 mmoles) of 3,5-bis-(trimethylsilyloxy)-6-methyl-as-triazine (IIb) and 3.8 g. (5.7 mmoles) of 2-deoxy-3,4,6-tri-O-p-nitrobenzoyl-α-D-arabinohexosyl bromide (III) (15) was heated at 70°; on stirring the mixture became clear and a gas [trimethyl silyl bromide?] was evolved. After heating over night at 90°, the semi-solid mixture was extracted three times with 25-ml. portions of chloroform. The chloroform extracts were evaporated to an oil *in vacuo* at 60°, and the residue digested with boiling absolute ethanol until the oil had solidified. Crystallization from acetic acid/water gave 1.8 g. (44%) of IVb, m.p. 151-153°.

Anal. Calcd. for $C_{31}H_{24}N_6O_{15}$: C, 51.67; H, 3.35; N, 11.66. Found: C, 50.91; H, 3.50; N, 11.42.

1-(2-Deoxy-D-arabinohexopyranosyl)-6-azathymine (Vb).

A mixture of 720 mg. (1 mmole) of the protected nucleoside (IVb), 2 ml. of 1 N sodium methoxide, and 25 ml. of dry methanol was stirred for 1 hour, at which time it was evaporated *in vacuo* at 60° to an oil. The latter was treated with 25 ml. of water, made acidic (pH 1) by the addition of concentrated hydrochloric acid, and extracted twice with 25-ml. portions of chloroform. The aqueous layer was evaporated to dryness *in vacuo* at 60° to an oil, which was reconcentrated twice with absolute ethanol to remove the water. The resulting oil was then extracted with 100 ml. of hot acetone and filtered. The crude nucleoside (Vb) which was obtained after the evaporation of the acetone, *in vacuo*, was crystallized from 2-propanol/dry ether, yielding 220 mg. (85%) of Vb, m.p. 228-230°, [α]²⁶_D -1.4° (c 0.81 water), λ max (0.1 N HCl), 261 mμ (log ε 3.93), λ min (0.1 N HCl), 234 mμ, λ max (pH 7 buffer), 255 mμ (log ε 3.92), λ min (pH 7 buffer) 225 mμ, λ max (0.1 N NaOH), 250 mμ (log ε 3.97), λ max (Nujol), 5.94 μ (C=O).

Anal. Calcd. for $C_{10}H_{15}N_3O_6$: C, 43.95; H, 5.53; N, 15.38. Found: C, 43.92; H, 5.32; N, 15.25.

REFERENCES

(1) Presented in part before the Division of Carbohydrate chemistry, 152nd National Meeting of the American Chemical Society, New York, N. Y., Sept. 1966. This work was supported in part by U. S. Public Health Service Grant No. CA 08095, from the National Cancer Institute.

(2) To whom enquiries should be addressed.

(3) G. Etzold and P. Langen, *Chem. Ber.*, **98**, 1988 (1965).

(4) M. Zimmerman, *Biochem. Biophys. Res. Commun.*, **16**, 600 (1964); T. A. Krenitsky, J. W. Mellors and R. K. Barclay, *J. Biol. Chem.*, **240**, 1281 (1965).

(5) W. W. Zorbach and G. J. Durr, *J. Org. Chem.*, **27**, 1474 (1962).

(6) W. W. Zorbach and S. Saeki, *ibid.*, **29**, 2018 (1964).

(7) W. W. Zorbach, H. R. Munson and K. V. Bhat, *ibid.*, **30**, 3955 (1965).

(8) J. Skoda, *Progress in Nucleic Acid Research*, **3**, 197 (1963); G. B. Elion and G. H. Hitching, *Advan. Chemotherapy*, **2**, 91 (1965).

(9) R. H. Hall, *J. Am. Chem. Soc.*, **80**, 1145 (1958).

(10) J. Gut, *Advan. Heterocyclic Chem.*, **1**, 204 (1963).

(11) T. Nishimura and I. Iwai, *Chem. Pharm. Bull. (Japan)* **12**, 353 (1963).

(12a) T. Y. Shen, W. V. Rugle and R. L. Buglanesi, *J. Heterocyclic Chem.*, **2**, 495 (1965); (b) A. Dipple and C. Heidelberger, *J. Med. Chem.*, **9**, 715 (1966); (c) G. L. Tong, W. W. Lee and L. Goodman, *J. Heterocyclic Chem.*, **3**, 226 (1966).

(13) J. Gut, *Coll. Czech. Chem. Commun.*, **23**, 1588 (1958).

(14) E. Wittenburg, *Z. Chem.*, **4**, 303 (1964).

(15) W. W. Zorbach and G. Pietsch, *Ann. Chem.*, **655**, 26 (1962).

(16) G. E. Hilbert and T. B. Johnson, *J. Am. Chem. Soc.*, 52, 2001, 4489 (1930).

(17) K. Y. Zee-Cheng and C. C. Cheng, *J. Org. Chem.*, 27, 976 (1962); J. Jonas and J. Gut, *Coll. Czech. Chem. Commun.*, 26, 2155 (1961).

(18) M. Horak and J. Gut, *ibid.*, 26, 1680 (1961).

(19) All melting points were determined using a Kofler hot stage. Ultraviolet absorption spectra were recorded by a Bausch and Lomb Spectronic 505 spectrophotometer. Infrared spectra were determined on a Perkin Elmer Infracord spectrophotometer. Analyses were performed by Micro-Tech Laboratories, Skokie, Ill.

(20) The synthesis of this compound (IIa) without isolation is reported in reference 12c.

(21) In one instance, the product was obtained as an amorphous solid, m.p. 114-117° after drying; this material gave an acceptable analysis and was converted into its crystalline form by crystallization from ethyl acetate.