The Synthesis and Properties of 9-β-D-Arabinofuranosyl-6-hydroxylaminopurine (1)

Alfredo Giner-Sorolla

Division of Cell Biochemistry, Sloan-Kettering Institute for Cancer Research

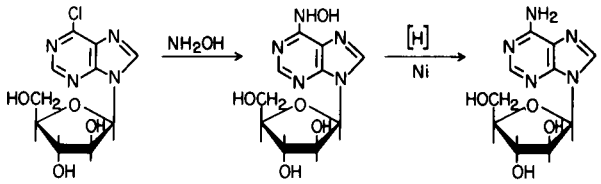
The adenosine analog 9-β-D-ribofuranosyl-6-hydroxylaminopurine (2) (HAPR) was found to possess marked antileukemic activity in mice (3) and inhibited the growth of *Streptococcus faecalis* (3c). Pharmacological studies of rats and dogs revealed that treatment with HAPR induced methemoglobinemia (4), a toxic manifestation also uncovered in clinical trials (5). Since hydrolysis of HAPR by ox heart adenosine deaminase to inosine occurs (6), it is likely that the liberation of hydroxylamine may be the cause of the hematoxicity noted above, although this requires confirmatory study (7). It is worth mentioning that prolonged administration of hydroxylamine to mice produced anemia and splenomegaly, and complete inhibition of spontaneous mammary tumors (8). However, hydroxylamine was ineffective against transplanted mouse tumors and leukemias (9). The preparation of 7-deaza-HAPR, an analog of 7-deazaadenine which is not affected by enzymatic deamination, has been reported (10).

The synthesis of 9-β-D-arabinofuranosyl-6-hydroxylaminopurine was undertaken to study its possible growth inhibitory activity and behavior towards adenosine deaminase. It is well known that derivatives with antitumor activity have been obtained by replacement of the ribosyl moiety of adenosine (11) or cytidine (12) by arabinosyl.

The synthesis of 9-β-D-arabinofuranosyl-6-hydroxylaminopurine (**2**) was achieved in 78% yield when 9-β-D-arabinofuranosyl-6-chloropurine (**1**) (13) was stirred with an excess of 0.6 *M* ethanolic hydroxylamine at 25° for 5 days. The usual method (14) for the preparation of a variety of hydroxylaminopurines and their ribosyl derivatives, namely refluxing of the appropriate chloro or methylmercapto compound with ethanolic hydroxylamine with or without chloride ions (14b), when applied to **1** gave a syrupy material from which compound **2** could not be isolated (**15**).

Attempts to prepare **2** by reaction of 9-β-D-arabinofuranosyl-6-mercaptopurine or its 6-methyl derivative (13) with ethanolic hydroxylamine in the presence of chloride ions led to a poor yield of **2**. Compound **2** was readily reduced to 9-β-D-arabinofuranosyladenine (**3**) (13) with Raney nickel.

Preliminary *in vitro* tests carried out by Dr. W. Kreis indicate that compound **2** is not a substrate for adenosine deaminase.



SCHEME 1

EXPERIMENTAL (16)

9-β-D-Arabinofuranosyl-6-hydroxylaminopurine (**2**).

A suspension of 9-β-D-arabinofuranosyl-6-chloropurine (**13**) (**1**, 3.40 g., 5.1 mmole) in 0.6 *M* ethanolic hydroxylamine (14a) (450 ml.) was stirred at 25°. The solid dissolved after several hours and later a copious precipitate appeared. Stirring was continued for a total of 5 days. The precipitate was filtered and washed with ethanol, suspended and stirred in cold water (40 ml.) and collected to yield 2.65 g. (78%) of white microneedles, m.p. 204° dec. An analytical sample was prepared by washing with cold water and ethanol, m.p. 206° dec, $[\alpha]_D^{25}$ +6° (*c* 0.5, water).

Compound **2** gave positive ferric chloride and phosphomolybdate tests, indicative of the hydroxylamino function; u.v. at *p*H 1, λ max 265 mμ (A_M 14.7 x 10^3), at *p*H 6.7 (phosphate buffer) λ max 267 mμ (A_M 10.4 x 10^3), pKa_1 = 3.7 (± 0.1). The solubility of **2** in water was 10.7 g./l. at 25° (± 1°).

No appreciable shift in u.v. spectra was observed when a 1% aqueous solution of **2** was boiled for 1 hour. A 1% aqueous solution of **2** when kept at 5° for 7 days did not show any significant change in u.v. spectrum.

Anal. Calcd. for $C_{10}H_{13}N_5O_5$: C, 42.40; H, 4.59; N, 24.73. Found: C, 42.21; H, 4.70; N, 24.55.

Reaction of 9-β-D-Arabinofuranosyl-6-mercaptopurine (**4**) or Its 6-Methyl Derivative (**5**) with Hydroxylamine.

9-β-D-Arabinofuranosyl-6-mercaptopurine (13) (**4**, 10 mg.) was suspended in 0.6 *M* ethanolic hydroxylamine (100 ml.) and

hydroxylamine hydrochloride (14b) and kept at 25° (15 mg.) for 15 days. After evaporation to dryness *in vacuo*, the residue appeared from paper chromatography and u.v. spectra, to consist of a mixture of starting material **4** and **2**, in a 2:1 ratio.

9-β-D-Ribofuranosyl-6-methylmercaptopurine (13) (**5**, 10 mg.) was added to a solution of 0.6 *M* ethanolic hydroxylamine (50 ml.) and hydroxylamine hydrochloride (15 mg.). The mixture was refluxed for 6 hours, and evaporated to dryness *in vacuo*. The residue appeared from paper chromatography and u.v. spectra to consist of a mixture of **5** and **2** approximately in a 3:1 ratio.

Treatment of **2** with Raney nickel.

Raney nickel (50 mg.) was added to a suspension of 9-β-D-arabinofuranosyl-6-hydroxylaminopurine (**2**, 5 mg.) in water (5 ml.) and the mixture was refluxed for 45 minutes. The filtrate, which no longer gave positive ferric chloride or phosphomolybdate tests, was evaporated to dryness under reduced pressure to yield a product which showed on paper chromatograms a single spot with R_fs and u.v. spectra identical to those of an authentic sample of 9-β-D-arabinofuranosyladenine (**3**) (spongoadenosine) (13).

Acknowledgment.

The author wishes to express his gratitude to Dr. Harry B. Wood, Jr., for a generous gift of spongoadenosine. He also wishes to thank Dr. A. Bendich and Dr. G. B. Brown for stimulating discussions. The skillful technical assistance of Mrs. C. Gryte is acknowledged.

REFERENCES

(1) This investigation was supported by funds from the National Cancer Institute (Grant No. CA 08748), The Atomic Energy Commission (Contract No. AT[30-1], 910), and aided by the Grant No. T-128F from the American Cancer Society.

(2a) A. Giner-Sorolla, L. Medrek, and A. Bendich, 150th National Meeting of the American Chemical Society, Atlantic City, New Jersey, September 1965, p. 5P; (b) A. Giner-Sorolla, *Galenica Acta* (Madrid), 19, 97 (1966); (c) P. K. Chang, *J. Med. Chem.*, 8, 884 (1965); (d) A. Giner-Sorolla, L. Medrek, and A. Bendich, *ibid.*, 9, 143 (1966).

(3a) J. H. Burchenal, J. J. Fox, A. Giner-Sorolla, and A. Bendich, XIth Congr. Int. Soc. Hemat., Sydney, Australia, 1966, p. 227; (b) J. H. Burchenal, M. Dollinger, J. Butterbaugh, D. Stoll, and A. Giner-Sorolla, *Biochem. Pharmacol.*, 16, 423 (1967); (c) A. Bloch, E. Mihich, C. A. Nichol, R. K. Robins, and R. H. Whistler, *Proc. Am. Ass. Cancer Res.*, (Abstract 25), 7, 7 (1966).

(4) F. S. Philips, personal communication.

(5) I. H. Krakoff and M. R. Dollinger, *Proc. Am. Ass. Cancer Res.*, (Abstract 186), 10, 47 (1969).

(6) M. Rockwell and M. H. Maguire, *Mol. Pharmacol.*, 2, 574 (1966).

(7a) E. Letsche, *Z. Physiol. Chem.*, 80, 412 (1912); (b) W. Heubner, *Arch. Exptl. Path. Pharmakol.*, 12, 239 (1913); (c) J. S. Colter and J. H. Quastel, *Arch. Biochem.*, 27, 368 (1950); (d) M. Kiese, *Pharmacol. Revs.*, 18, 1091 (1966).

(8) R. S. Yamamoto, E. K. Weisburger and J. Korzis, *Proc. Soc. Exptl. Biol. Med.*, 124, 1217 (1967).

(9) G. S. Tarnowski, W. Kreis, F. A. Schmidt, J. G. Capuccino and J. H. Burchenal, *Cancer Res.*, 26, (Part 2), 1279 (1966).

(10) B. C. Hinshaw, R. K. Robins, and L. B. Townsend, *J. Heterocyclic Chem.*, 5, 885 (1968).

(11a) J. J. Brink and G. A. LePage, *Cancer Res.*, 24, 312 (1964); (b) J. J. Brink and G. A. LePage, *Can. J. Biochem.*, 42, 1 (1965); (c) C. G. Smith in "The Control of Growth Processes by Chemical Agents," Proc. 3rd Inter. Pharmacol. Meeting, Sao Paulo, Brazil A. D. Welch, Ed., Pergamon Press, 1966, p. 33.

(12a) R. Talley and V. K. Viatkevicius, *Blood*, 21, 352 (1963); (b) E. S. Henderson and P. J. Burke, *Proc. Am. Assoc. Cancer Res.*, 6, 26 (1965); (c) R. W. Carey and R. R. Ellison, *Clin. Res.*, 13, 337 (1965).

(13) E. J. Reist, A. Benitez, L. Goodman, B. R. Baker and W. W. Lee, *J. Org. Chem.*, 27, 3274 (1962).

(14a) A. Giner-Sorolla and A. Bendich, *J. Am. Chem. Soc.*, 80, 3932 (1958); (b) A. Giner-Sorolla, S. A. O'Bryant, J. H. Burchenal and A. Bendich, *Biochemistry* 5, 3057 (1966); (c) A. Giner-Sorolla, C. Nanos, M. R. Dollinger, J. H. Burchenal and A. Bendich, *J. Med. Chem.*, 11, 52 (1968); (d) A. Giner-Sorolla, S. A. O'Bryant, C. Nanos, M. R. Dollinger, A. Bendich and J. H. Burchenal, *J. Med. Chem.*, 11, 521 (1968); *cf.* also Reference 2.

(15) A similar observation was recently made (A. Giner-Sorolla, *et al.*, *Amer. Chem. Soc. Regional Meeting (Metrochem.)*, Abstracts of Papers, p. 35, New York, N. Y., May (1969) in the synthesis of 6-hydroxylaminopurine 3-oxide from purine-6-sulfonate 3-oxide and ethanolic hydroxylamine, feasible only when carried out at 25°.

(16) Ultraviolet absorption spectra were determined with a Beckman spectrophotometer, Model DU. Ascending paper chromatography was run on Whatman No. 1 paper in the following solvent systems: concentrated aqueous ammonia-water-isopropyl alcohol (10:20:70); 1-butanol-water-acetic acid (50:25:25); and 1 *M* ammonium acetate-ethanol (35:70). The determination of melting points was carried out with a Thomas-Hoover melting point apparatus and the temperatures were corrected. Analyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Michigan.

Received March 21, 1969 New York, New York 10021