tions followed by lyophilization gave 330 mg (64.7%) of the product: mp 135-140° dec; ir max 1710 (ureido, C=O), 1220 (P=O), and 1060 cm^{-1} (POC); nmr δ 1.18 (d, 6, J = 6 Hz, CH_3), 4.78-3.78 (m, 6, 3'-H, 2'-H, 4'-H, 5'-H, =CH-), 6.10 (d, 1, J = 5.5 Hz, 1'-H), 8.67 (s, 1, 2-H), 8.73 (s, 1, 8-H), 9.18 (s, NH), and 9.33 ppm (s, NH). For analysis, the product was converted to the Ba salt by adjusting the aqueous solution to pH 7.5 with saturated $Ba(OH)_2$ and by precipitating with acetone, mp 245-250° dec. *Anal.* ($C_{14}H_{19}N_6O_8PBa \cdot 3H_2O$) C, H, N, P. This compound was also prepared by method A (22.3%) and method B (47.5%).

***N*-(Purin-6-ylcarbamoyl)allylamine Ribonucleoside 5'-Phosphate (2d).** This compound was prepared by method B in 47.8% yield: mp 133-134° dec; ir max 1710 (ureido, C=O), 1220 (P=O), and 1040 cm^{-1} (POC); nmr δ 3.93-4.20 (m, 5, CH_2, 4'-H, 5'-H), 4.70 (m, 1, 3'-H), 5.13-5.47 (m, 3, NCH_2, -CH=), 6.15 (d, 1, J = 6 Hz, 1'-H), 8.77 (s, 1, 2-H), and 8.87 ppm (s, 1, 8-H). *Anal.* ($C_{14}H_{19}N_6O_8P \cdot 1.5H_2O$) C, H, N, P.

***N*-(Purin-6-ylcarbamoyl)isoamylamine Ribonucleoside 5'-Phosphate (2e).** This compound was prepared by method B in 47.5% yield: ir max 1710 (ureido, C=O), 1230 (P=O), and 1030 cm^{-1} (POC); nmr δ 0.92 (d, 6, J = 6 Hz, CH_3), 1.45 (m, 3, CH_2, -CH=), 3.29 (h, 2, J = 6 Hz, J = 3 Hz, NCH_2), 4.23-3.92 (m, 4, 5'-H, 4'-H, 2'-H), 4.65 (q, 1, J = 5.5 Hz, J = 3 Hz, 3'-H), 6.16 (d, 1, J = 5.5 Hz, 1'-H), 8.8 (s, 1, 2-H), and 8.92 ppm (s, 1, 8-H). The analytical sample was purified from a cellulose column (neutral, Whatman) using *i*-PrOH-H_2O-concentrated NH_4OH (7:2:1), mp 151-155° dec. *Anal.* ($C_{16}H_{23}N_6O_8P \cdot 2NH_4$) C, H, P; N: calcd, 22.66; found, 20.45.

Enzymatic Hydrolysis. (a) 5'-Nucleotidase. The substrate (3.0 μmol) in H_2O (20 μl) was incubated with 1 *M* Tris-HCl, pH 9.0 (20 μl), and 5'-nucleotidase (25 μl, 10 mg/ml, Sigma Chemical Co.) at 37° for 3 hr.

(b) Intestinal Alkaline Phosphatase. The substrate (3.0 μmol) in H_2O (20 μl) was incubated with 1 *M* Tris-HCl, pH 8.0 (20 μl), and intestinal alkaline phosphatase (25 μl, 10 mg/ml, Worthington Biochemical Corp.) at 25° overnight.

(c) Acid Phosphatase. The substrate (3.0 μmol) in H_2O (20 μl) was incubated with 0.15 *M* NaOAc buffer, pH 5.5 (20 μl), and wheat germ acid phosphatase (25 μl, 10 mg/ml, Worthington Biochemical Corp.) at 25° overnight.

(d) Venom Phosphodiesterase. The substrate (3.0 μmol) in H_2O (20 μl) was incubated with 1 *M* Tris-HCl and 0.01 *M* $MgSO_4$, pH 8.8 (20 μl), and venom phosphodiesterase (25 μl, 10 mg/ml, Ross Allen's Reptile Institute) at 37° for 3 hr.

In all cases, the incubation mixtures, analyzed by paper electrophresis and paper chromatography, revealed that the nucleotides were converted into the nucleosides. There was no unchanged nucleotide.

Acknowledgment. We are thankful to Dr. M. P. Schweizer for providing us with a sample of natural PCTR 5'-P. We wish to thank Drs. A. Mittelman, G. P. Murphy, and E. D. Holyoke for their keen interest and encouragement in this work. This research was supported from U. S. Public Health Service Grants CA 14185 and 2-MO1-RR00262-07 and the NSF Grant GB 29394.

References

(1) G. B. Chheda, R. H. Hall, D. I. Magrath, J. Mozejko, M. P. Schweizer, L. Stasiuk, and P. R. Taylor, *Biochemistry*, 8, 3278 (1969).
(2) M. P. Schweizer, G. B. Chheda, R. H. Hall, and L. Baczynskyj, *ibid.*, 8, 3283 (1969).
(3) G. B. Chheda, *Life Sci.*, 8, 979 (1969).
(4) S. Takemura, M. Murakami, and M. Miyazaki, *J. Biochem. (Tokyo)*, 65, 553 (1969).
(5) H. Ishikura, Y. Yamada, K. Murao, M. Saneyoshi, and S. Nishimura, *Biochem. Biophys. Res. Commun.*, 37, 990 (1969).
(6) F. Harada and S. Nishimura, *Biochemistry*, 11, 301 (1972).
(7) M. P. Schweizer, K. McGrath, and L. Baczynskyj, *Biochem. Biophys. Res. Commun.*, 40, 1046 (1970).
(8) G. B. Chheda and C. I. Hong, *J. Med. Chem.*, 14, 748 (1971).
(9) C. I. Hong, G. B. Chheda, S. P. Dutta, E. O. Curtis, and G. L. Tritsch, *ibid.*, 16, 139 (1973).
(10) W. H. Dyson, C. M. Chen, S. M. Alam, R. H. Hall, C. I. Hong, and G. B. Chheda, *Science*, 170, 328 (1970).
(11) W. H. Dyson, R. H. Hall, C. I. Hong, S. P. Dutta, and G. B. Chheda, *Can. J. Biochem.*, 50, 237 (1972).
(12) J. Baddiley and A. R. Todd, *J. Chem. Soc. (London)*, 648 (1947).
(13) M. Yoshikawa, T. Kato, and T. Tanenishi, *Tetrahedron Lett.*, 50, 5065 (1967).
(14) K. Imai, S. Fujii, K. Takanohashi, Y. Furukawa, T. Masuda, and M. Honjo, *J. Org. Chem.*, 34, 1547 (1969).
(15) G. M. Tener, *J. Amer. Chem. Soc.*, 83, 159 (1961).
(16) P. C. Crofts, I. M. Downie, and R. B. Heslop, *J. Chem. Soc. (London)*, 3673 (1960).

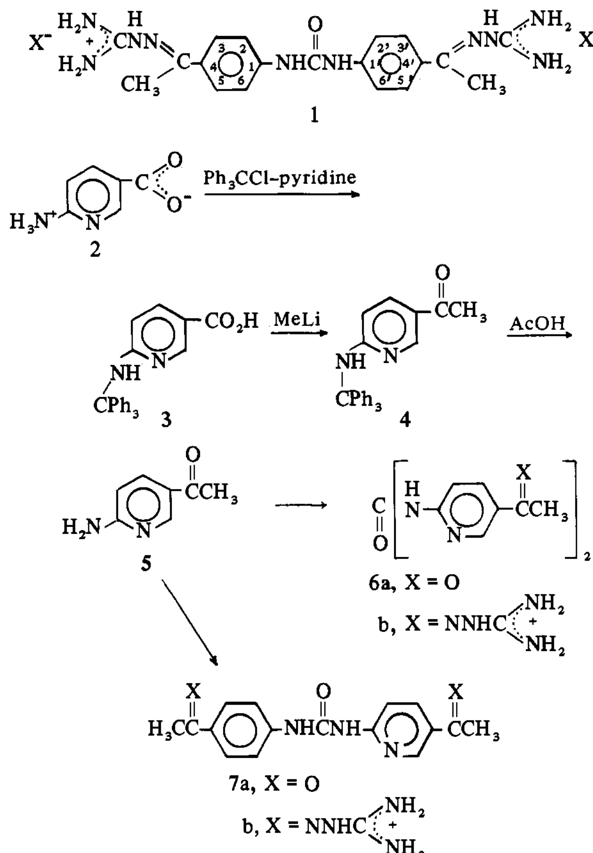
Guanylhydrazones with Potential Antileukemic Activity. 1. Aza Analogs of 4,4'-Diacetyldiphenylurea Bis(guanylhydrazone)

W. Korytnyk,* A. C. Ghosh, N. Angelino, and C. Dave

Department of Experimental Therapeutics, Roswell Park Memorial Institute, Buffalo, New York 14203. *Received January 31, 1973*

The finding of significant antitumor effect for methylglyoxal bis(guanylhydrazone) (Me-G)[1,2] and the subsequent use of the compound in the clinic were followed by considerable research efforts in which a number of closely analogous compounds were synthesized, none of which proved to be as active as the parent compound. More recently, it has been found that 4,4'-diacetyldiphenylurea bis(guanylhydrazone) (DDUG, **1**) has a marked inhibitory activity against a wide spectrum of tumors and is the most potent bis(guanylhydrazone) against L1210 tumors.[3,4] This inhibitor was first synthesized by Marxer, who has also prepared several closely related compounds.[4] In contrast to Me-G congeners, these analogs also exhibit marked antitumor effects. The requirement for less stringent structural specificity in this series[5,6] may be related to passive diffusion as the mechanism of cellular transport in the case of DDUG and its derivatives.[7]

We have undertaken the synthesis of two aza analogs of DDUG, **6b** and **7b**, which differ from **1** by replacement of carbon atoms in the phenyl ring (both 2 and 2' in **6b**, but



only 2 in **7b**) with N. It was hoped that these analogs would either show an improved therapeutic index or else provide a type of selectivity different from that of DDUG by virtue of their enhanced chelating properties resulting from the hetero N ortho to the urea moiety.

Fortuitously, the scheme of synthesis that was followed provides an analog of nicotinamide, 3-acetyl-6-aminopyridine (**5**), also of potential interest as an antineoplastic agent. It was shown earlier that 6-aminonicotinamide is a potent nicotinamide antagonist and has antitumor activity.[8] 3-Acetylpyridine is a well-known nicotinamide antagonist. Thus, compound **5** combines the structural features of both antagonists.

Reaction of 6-aminonicotinic acid with methyllithium was not successful, presumably because of the insolubility of the former; but after protection of the amino group with a triphenylmethyl (trityl) group (**3**), the reaction gave the expected methyl ketone **4** in a satisfactory yield, as well as some trityl alcohol as a by-product. The *N*-oxide of 4 has also been prepared. Detritylation of both compounds proceeded smoothly with 80% acetic acid, giving **5** and its *N*-oxide, respectively.

Considerable difficulties were experienced in obtaining the symmetrical urea derivative **6a**, since the basicity of the amino group in **5** is decreased by electron-withdrawing groups ortho and para to it. Among the many methods tried, only a prolonged passage of phosgene into a pyridine solution of **5** was successful in yielding the desired urea derivative **6a**. The unsymmetrical urea derivative **7b** was obtained by condensing with **5** the isocyanate derived from *p*-aminoacetophenone. Both bis(guanylhydrazones) **6b** and **7b** were obtained by condensing the keto urea precursors with aminoguanidine sulfate and were crystallized as the hydrobromide salts.

Biological Activity. Compounds prepared in this study were tested for inhibition of the growth of mouse mammary adenocarcinoma (TA-3) cells in tissue culture. The cells were grown in RPMI medium 2640 containing 8×10^{-6} *M* nicotinamide. DDUG (**1**) inhibited growth by 50% at 6.2×10^{-6} *M*, whereas the aza analogs **6b** and **7b** inhibited at 6.6×10^{-6} and 7.3×10^{-6} *M*, respectively. The intermediate ureas **6a** and **7a** were inactive in this system. Although 6-aminonicotinamide did inhibit the cells at ID_{50} 4.5×10^{-6} *M*, 3-acetyl-6-aminopyridine (**4**) did not inhibit at 1×10^{-4} *M*.

Since both **6b** and **7b** were available in limited quantity, only preliminary information regarding their possible antileukemic activities could be obtained. Groups of three female DBA/2J mice inoculated ip with 1×10^6 leukemia L 1210 cells on day 0 were subsequently treated ip with 30 mg/kg daily with **6b** on days 1 and 2 or with **7b** on days 1-4. In comparison with control animals (average survival 8.5 days), a 29% increase in survival occurred with **6b** and a 33% increase with **7b**; with DDUG, at 30 mg/kg ip on days 1-4, the increase of survival was 270%.

The antileukemic activity of DDUG has been found to correlate with the ability of DDUG to inhibit DNA biosynthesis catalyzed by DNA polymerase; this inhibition is presumably caused by binding to DNA template.[9] On the basis of this finding, **6b** and **7b** were also tested for their ability to inhibit the reaction of L1210 DNA polymerase in a cell-free system using Bollum's procedure.[10] A 50% inhibition of the reaction of DNA polymerase occurred with **7b** at 1.6×10^{-4} *M* concentration, while the corresponding concentration with DDUG was 0.6×10^{-4} *M*. No such information could be obtained for **6b**, however, because of the inadequate solubility of the compound. The present results indicate that replacement of one or both of the benzene rings in DDUG with a heterocyclic ring, in this instance a pyridine ring, does not abolish the biological activity of the parent compound.

Experimental Section

Melting points are capillary and are uncorrected. All compounds had ir and nmr spectra consistent with assigned structures. Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values.

6-(Tritylamino)nicotinic Acid (3). 6-Aminonicotinic acid (**2**) hydrochloride (5.0 g, 29.5 mmol), obtained on acid hydrolysis of commercial 6-aminonicotinamide, and trityl chloride (30 g, 104 mmol) in pyridine (50 ml) were heated on a steam bath for 30 min and then kept at room temperature overnight. After concentration *in vacuo*, the oily material was treated with H_2O, extracted with $CHCl_3$, and dried ($MgSO_4$). Removal of the solvent from the filtrate left a solid, containing some trityl alcohol. The last was removed by washing the solid with a mixture of Et_2O and petroleum ether (bp 30-60°) and finally with $CHCl_3$-Et_2O. The residual solid (7.0 g) was combined with material (0.6 g) crystallized from the washings, giving a total yield of 69%: mp 273-276° (from MeOH-$CHCl_3$). *Anal.* ($C_{25}H_{20}N_2O_2$) C, H, N.

2-(Tritylamino)-5-acetylpyridine (4). 6-(Tritylamino)nicotinic acid (5 g, 13.8 mmol) was dissolved in THF (300 ml, freshly distilled over $LiAlH_4$) and cooled in ice. While N_2 was passed in and the solution was stirred, CH_3Li solution (4.3% in Et_2O, 10 ml, 13.9 mmol) was added dropwise. After the reaction mixture was stirred for 5 hr, a few drops of 10% NH_4Cl solution were added, followed by H_2O (50 ml) and Et_2O (200 ml). The organic layer was separated, dried ($MgSO_4$), and evaporated, giving an oily solid (4.58 g). Tlc (silica gel, Et_2O) showed one major spot of **4** (R_f 0.8) and two minor ones (R_f 0.99 and 0.5). The major component was separated from trityl alcohol (R_f 0.99, 38 mg) and 2-(tritylamino)pyridine-5-(1-ethanol) (R_f 0.5, 0.85 g) by column chromatography on silica gel (developed by petroleum ether and Et_2O-$CHCl_3$). The major compound was crystallized from Et_2O: yield 3.5 g (73%); mp 160-161°. *Anal.* ($C_{26}H_{22}N_2O$) C, H, N.

***N*-Oxide of 4.** Compound **4** (300 mg, 0.79 mmol) was allowed to react with *m*-chloroperbenzoic acid (200 mg, 1.16 mmol) in $CHCl_3$ (50 ml) at room temperature for 12 hr. After washing successively with $NaHSO_3$ and $NaHCO_3$ solutions and H_2O, the $CHCl_3$ layer was dried and evaporated. The oily solid was recrystallized from a mixture of $CHCl_3$ and petroleum ether; the yield was 260 mg (84%), mp 219-221°. *Anal.* ($C_{26}H_{22}N_2O_2$) C, H, N.

3-Acetyl-6-aminopyridine (5). A solution of the trityl derivative **4** (4.5 g, 11.9 mmol) in AcOH (80%, 60 ml) was heated on a steam bath for 30 min and then was allowed to stand at room temperature overnight. The solution was evaporated, and trityl alcohol was crystallized from 50% AcOH. The faintly colored residue from the mother liquor was recrystallized from Et_2O: yield 1.52 g (93%); mp 89-90°. *Anal.* ($C_7H_8N_2$) C, H, N.

The *N*-oxide of **5**, prepared by detritylation of the *N*-oxide of **4** by heating with AcOH as described, was converted to the hydrochloride with 6 *N* HCl: mp 190-191° (from Me_2CO-H_2O). *Anal.* ($C_7H_9ClN_2O_2$) C, H, N.

2,3-Bis(5-acetyl-2-pyridyl)urea (6a). Phosgene was passed through a solution of 2-amino-5-acetylpyridine (850 mg) in pyridine (50 ml) for 4 hr. After flushing the solution with N_2 (some solid material separated out at this stage), pyridine was evaporated, giving a solid. Tlc (silica gel, EtAc) indicated the disappearance of **5** (R_f 0.35) and the presence of the main product (R_f 0.6) and a by-product (R_f 0.45). The solid was extracted with 1:1 Me_2CO-MeOH, which dissolved the by-product, leaving 252 mg of the solid. After further washing with Me_2CO-MeOH, the solid was recrystallized from MeOH-THF and finally from a large volume of EtOH: mp 260-261°. The filtrates were combined and concentrated, giving 92 mg of impure compound which was taken up in $CHCl_3$, extracted with 10% $NaHCO_3$ solution and H_2O, and dried ($MgSO_4$). After treatment with charcoal, 63 mg of pure **5** was obtained upon filtration and concentration, raising the yield to 315 mg (33%); molecular ion at *m/e* 298. *Anal.* ($C_{15}H_{15}N_4O_3$) C, H, N.

1-(4-Acetylphenyl)-3-(5-acetyl-2-pyridyl)urea (7a). 4-Isocyanatoacetophenone was prepared by the interaction of phosgene with *p*-aminoacetophenone in freshly distilled EtOAc under standard conditions. The isocyanate was crystallized from CCl_4: mp 74-79°. It had

the characteristic peak at 2265 cm^{-1}, but satisfactory elemental analysis could not be obtained. A solution of this product (0.592 g, 3.67 mmol) in dry PhH (3.5 ml) was added to **5** (0.50 g, 3.67 mmol) suspended in PhH (3.5 ml) containing Et_3N (1 ml). After stirring overnight with moisture excluded, the reaction mixture was filtered and washed (H_2O). The yield was 0.565 g (51%). The compound was recrystallized several times from hot DMF–MeOH, and the crystals were washed with Et_2O: mp 247–253° (softens at 243°). *Anal.* ($C_{16}H_{15}N_3O_3$) C, H, N.

1,3-Bis(5-acetyl-2-pyridyl)urea Bis(guanylhydrazone) (6b) Hydrobromide. To the symmetrical urea derivative **6a** (90 mg, 0.302 mmol) in DMF (1 ml) were added 16% HBr (0.5 ml) and aminoguanidine sulfate (93 mg, 0.755 mmol). The solution was heated to boiling, when a light brown precipitate formed, yielding 186 mg (94%) of **6b**. Considerable difficulty was experienced in crystallizing the material, since it precipitated as a gel from hot DMF: mp 230–235° dec, with shrinking at 217°. *Anal.* ($C_{17}H_{25}Br_3ON_{12}$) C, H, N.

1-(4-Acetylphenyl)-3-(5-acetyl-2-pyridyl)urea Bis(guanylhydrazone) (7b) Hydrobromide. To the unsymmetrical urea derivative **7a** (0.167 g, 0.563 mmol) in a solution of DMF (1.5 ml), MeOH (1.8 ml), H_2O (0.5 ml), and 16% HBr (0.5 ml) was added aminoguanidine sulfate (0.194 g, 1.58 mmol) and the mixture was heated. The product precipitated immediately. After standing for 3 hr and filtration, it was washed with Me_2CO, yielding 0.30 g (85%). The product was purified by repeatedly dissolving it in DMF containing HBr and precipitating it with either MeOH or isobutyl alcohol. Finally the product was washed with H_2O and then with Me_2CO. *Anal.* ($C_{18}H_{25}Br_2N_{11}O \cdot 2H_2O$) C, H, N.

Acknowledgment. This study was supported in part by research grants (CA-08793 and CA-13038) from the National Cancer Institute, U. S. Public Health Service, and Institutional Research Grant IN 54 J-20 from the American Cancer Society. Testing data on the mammary adenocarcinoma (TA-3) cells were provided by Dr. M. T. Hakala and Miss A. Mulhern. We thank Dr. Enrico Mihich for his encouragement and interest in this program.

References

(1) B. L. Freedlander and F. A. French, *Cancer Res.*, 18, 360 (1968).
(2) (a) E. Mihich, *Arch. Ital. Patol. Clin. Tumori*, 8, 153 (1965); (b) E. Mihich in "Antineoplastic and Immunosuppressive Agents," A. Sartorelli and D. Johns, Ed., Springer Verlag, New York, N. Y., in press.
(3) (a) E. Mihich and J. Gelzer, *Cancer Res.*, 28, 553 (1968); (b) E. Mihich and A. I. Mulhern, *ibid.*, 28, 354 (1968).
(4) A. Marxer, *Experientia*, 23, 173 (1967).
(5) A. Marxer and J. Gelzer, *Proc. Int. Congr. Chemother., 5th*, 2, 277 (1967).
(6) (a) E. Mihich and M. T. Hakala, *Proc. Amer. Ass. Cancer Res.*, 9, 48 (1968); (b) C. Dave, J. Ehrke, J. Soucek, and E. Mihich, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, 30, 628 (1971).
(7) (a) M. T. Hakala, *Biochem. Pharmacol.*, 20, 81, 1971; (b) C. Dave and E. Mihich, *ibid.*, 21, 2681 (1972).
(8) D. M. Shapiro, L. S. Dietrich, and M. E. Shils, *Cancer Res.*, 17, 600 (1957).
(9) (a) C. Dave, J. Ehrke, and E. Mihich, *Proc. Amer. Soc. Cancer Res.*, 12, 40 (1971); (b) C. Dave, J. Ehrke, J. Soucek, and E. Mihich, *Proc. Int. Congr. Chemother., 7th*, 2, B-1.4/12 (1971).
(10) F. J. Bollum, *Proc. Nucl. Acid Res.*, 296 (1966).

Book Reviews

Synthetic Methods of Organic Chemistry. Vol. 26. Edited by W. Theilheimer. S. Karger, Basel, Switzerland. 1972. 576 pp. 15 × 22.5 cm. $79.80.

This new volume of Theilheimer introduces the sixth series of this important work much to the appreciation of chemists engaged in organic synthesis. Almost 1000 new references to papers published between 1969 and 1971 are presented in the usual systematic manner. New references to material in the preceding series have been included and the index also contains additional and revised entries to former volumes. The useful survey on trends in synthetic organic chemistry appears for 1972 in this volume.

In an informal survey of this department, it was found that a considerable number of graduate students engaged in organic synthesis did not use Theilheimer, primarily because of difficulty in mastering the classification system. Each volume contains explanatory notes on classification and actual examples are given in volume 2, which, unfortunately, is now out of print and consequently may be unavailable in some newer libraries. Thus, the editor may give some consideration to providing a more extensive discussion of the classification system, including examples, in the next volume. It would be a pity for anyone engaged in organic synthesis not to have complete command of this worthwhile series.

Department of Medicinal Chemistry
College of Pharmacy
University of Minnesota
Minneapolis, Minnesota 55455
Kevin H. Bell

Regulation of Purine Biosynthesis. ACS Monograph 170. By J. Frank Henderson. American Chemical Society, Washington, D. C. 1972. 303 pp.

The ACS Monograph Series is intended to serve two purposes: first, to provide a thorough treatment of a selected area for persons working in unrelated fields so that they may correlate their own work with a larger area; second, to stimulate further research in the specific field treated. Dr. Henderson's monograph should serve both purposes admirably. The worthwhileness of surveying such a narrow field as the regulation of purine biosynthesis might be open to question had not the author done such an excellent job of relating the subject to such clearly important and diverse topics as the whole field of intermediary metabolism, human diseases resulting from aberrant rates of purine synthesis, and the mechanism of action of such important drugs as 6-mercaptopurine. "Regulation" is defined broadly to include all factors which actually or potentially affect the rate of purine biosynthesis or constituent parts of the pathway.

The first chapter is devoted to an overview of purines in nature and an introduction to the mechanisms of regulation of the *de novo* pathway. The second chapter then discusses in detail the *de novo* pathway, and the third chapter, only four pages long, describes the properties of the individual enzymes that carry out each of the ten steps of the pathway. The fourth chapter on substrate concentrations, which includes lengthy sections on related subjects such as the pathways of glycine synthesis and their regulation, the folate metabolizing enzymes, and the importance of δ-aminolevulinate and methionine and which is actually almost one-half of the text, illustrates the complexity of the subject and points up the fact that our current knowledge, as Dr. Henderson puts it, "represents merely the top of an iceberg." In chapter five the author points out that the physiological significance of end-product inhibition of purine biosynthesis *de novo*, considered by many to be *the* control mechanism, is, in fact, not clear today, particularly in mammalian cells *in vivo*. The paucity of information on the subject of chapter six–regulation of enzyme amount and genetic regulation–is more widely appreciated. The chapter on the effects of drugs on purine biosynthesis is not as thorough and complete in its coverage as most of the preceding chapters, perhaps because this subject has already been reviewed extensively in the literature and is really not a major concern of this work. Even so, information very pertinent to the mechanism of