a preparative thin layer plate using $CHCl_3$–MeOH (9:1) as the eluent. The major band was eluted from the silica gel with MeOH and the solution was evaporated to dryness *in vacuo*. The residue (137 mg) was dissolved in EtOAc (3 ml) and filtered through dry Celite, and the filtrate was refrigerated until crystallization was complete. The crystals were collected by filtration and recrystallized from EtOAc; yield 59 mg (20%), mp 174–176°, $[\alpha]^{23}D$ −40.9 ± 0.1° (*c* 0.86 g/100 ml of MeOH). Thin layer chromatography using $CHCl_3$–MeOH (4:1) as the eluent showed a single spot.

Anal. Calcd for $C_{11}H_{13}FN_4O_4$: C, 46.49; H, 4.61; N, 19.70. Found: C, 46.55; H, 4.74; N, 19.36.

6-Methyl-9-β-D-xylofuranosylpurine (14).—Crude **14** (646 mg) was triturated with anhydrous ether. The insoluble gum that formed was dissolved in EtOH (1 ml), crystallization was initiated by scratching, and the solution was diluted with additional EtOH and refrigerated overnight. The crystals (180 mg) that formed were collected by filtration, washed (EtOH, Et_2O), and recrystallized from EtOH (5 ml) to give the pure β anomer: yield 117 mg (24%), mp 162–163° (Mel-Temp), $[\alpha]^{25}D$ −53.6 ± 0.1° (*c* 1.03 g/100 ml of MeOH). Thin layer chromatography using $CHCl_3$–MeOH (4:1) as the eluent showed a single spot.

Anal. Calcd for $C_{11}H_{14}N_4O_4$: C, 49.62; H, 5.31; N, 21.05. Found: C, 49.91; H, 5.32; N, 21.04.

A sample of chromatographically homogeneous α anomer was isolated as an oil by repeated preparative thin layer chromatography using $CHCl_3$–MeOH (3:1) as the eluent; $[\alpha]^{25}D$ −10.4 ± 0.3° (*c* 1.01 g/100 ml of MeOH).

Acknowledgment.—The authors are indebted to Dr. W. J. Barrett and members of the Analytical and Physical Chemistry Division of Southern Research Institute for the spectral and microanalytical data reported, to Mrs. Martha Thorpe for her help in the interpretation of the pmr spectra, and to Mrs. Margaret H. Vail for the cytotoxicity data reported.

The Synthesis and Properties of 2,6-Dihydroxylaminopurine and Its 9-β-D-Ribofuranosyl Derivative[1]

A. GINER-SOROLLA, C. NANOS, J. H. BURCHENAL, M. DOLLINGER, AND A. BENDICH

Divisions of Biological Chemistry and Drug Resistance, Sloan-Kettering Institute for Cancer Research, and Sloan-Kettering Division, Graduate School of Medical Sciences, Cornell University Medical College, New York, New York 10021

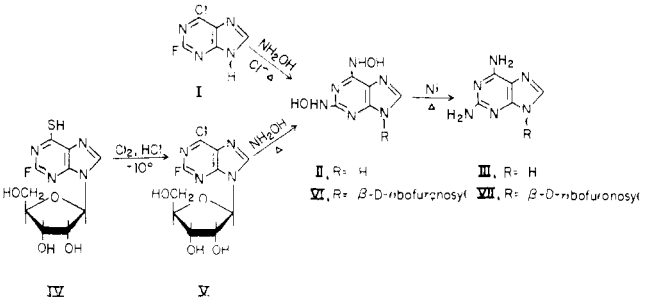
Received August 12, 1967

Syntheses of 2,6-dihydroxylaminopurine and its 9-β-D-ribofuranosyl derivative from the corresponding 6-chloro-2-fluoro compounds and hydroxylamine are described. The dihydroxylaminopurines were converted into the corresponding diamines by reduction with Raney nickel. Several mouse leukemias and Ridgeway osteogenic sarcoma were inhibited by 2,6-dihydroxylaminopurine, and the pattern of activity in various resistant lines of mouse leukemia suggested that this compound may exert its antileukemic effect by a mechanism similar to that of 6-mercaptopurine.

The biological activity shown by the adenine analog, 6-hydroxylaminopurine,[2] and the marked inhibitory effect of its 9-β-D-ribosyl derivative on mouse leukemias[3] stimulated our interest in the synthesis of the corresponding 2,6-dihydroxylamino compounds. These derivatives can be considered as analogs of 2,6-diaminopurine, the first purine reported to exert an inhibitory effect on mouse leukemia.[4] A bishydroxylamino derivative, dihydroxyurea, has recently been found to be a powerful inhibitor of DNA biosynthesis in HeLa cells[5] and to be active against several experimental tumors.[6] Related hydroxylamine derivatives induce chromosomal aberrations in cultured mammalian cells and exert a direct degradative action on DNA.[7]

The bishydroxylamino derivatives were obtained by interaction of 6-chloro-2-fluoropurine or its 9-ribosyl derivative with ethanolic hydroxylamine. Reaction of 6-chloro-2-fluoropurine[8] (I) (Scheme I) with ethanolic

SCHEME I



hydroxylamine in the presence of chloride ions afforded 2,6-dihydroxylaminopurine (II) in 70% yield, which

(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 Grant No. T-128F from the American Cancer Society, Grant T45, an Ethel A. Shaffer Memorial Grant for Cancer Research from the American Cancer Society, and U. S. Public Health Service Fellowship No. 1-F3-CA-32,812.

(2) (a) A. Giner-Sorolla and A. Bendich, *J. Am. Chem. Soc.*, **80**, 3932 (1958); (b) A. Giner-Sorolla, Ph.D. Thesis, Cornell University, Ithaca, N. Y.; *Dissertation Abstr.*, **20**, 1148 (1959); (c) A. C. Sartorelli, A. L. Bieber, P. K. Chang, and G. A. Fischer, *Biochem. Pharmacol.*, **13**, 507 (1964); (d) P. F. Pecora and M. E. Balis, *ibid.*, **13**, 1071 (1964); (e) A. Giner-Sorolla, *Galenica Acta* (Madrid), **19**, 97 (1966).

(3) (a) A. Giner-Sorolla, L. Medrek, and A. Bendich, Abstracts of the 150th National Meeting of the American Chemical Society, Atlantic City, N. J., Sept 1965, p 5P; (b) A. Giner-Sorolla, L. Medrek, and A. Bendich, *J. Med. Chem.*, **9**, 143 (1966); (c) J. H. Burchenal, J. J. Fox, A. Giner-Sorolla, and A. Bendich, XIth Congress of the International Society of Hematology, Sydney, Australia, 1966, p 227; (d) J. H. Burchenal, M. Dollinger, J. Butterbaugh, D. Stoll, and A. Giner-Sorolla, *Biochem. Pharmacol.*, **16**, 423 (1967).

(4) (a) G. B. Elion and G. H. Hitchings, *J. Biol. Chem.*, **187**, 511 (1950); (b) A. Bendich and G. B. Brown, *ibid.*, **176**, 1471 (1948); (c) J. H. Burchenal, A. Bendich, G. B. Brown, G. B. Elion, G. H. Hitchings, C. P. Rhoads, and C. C. Stock, *Cancer*, **2**, 119 (1949); (d) L. L. Bennett, Jr., J. E. Skipper, C. C. Stock, and C. P. Rhoads, *Cancer Res.*, **15**, 485 (1955).

(5) (a) C. W. Young and S. Hodas, *Science*, **146**, 1172 (1964); (b) C. W. Young, G. Schochetman and D. A. Karnofsky, *Cancer Res.*, **27**, 526 (1967); (d) C. W. Young, G. Schochetman, S. Hodas, and M. E. Balis, *ibid.*, **27**, 535 (1967).

(6) G. S. Tarnowski, W. Kreis, F. A. Schmid, J. G. Capuccino, and J. H. Burchenal, *ibid.*, **26**, Part 2, 1279 (1966).

(7) (a) E. Borenfreund, M. Krim, and A. Bendich, *J. Nat. Cancer Inst.*, **32**, 667 (1964); (b) A. Bendich, E. Borenfreund, G. C. Korngold, M. Krim, and M. E. Balis, *Ist. Lombardo Acad. Sci. Letters*, 214 (1963).

(8) J. A. Montgomery and K. Hewson, *J. Am. Chem. Soc.*, **82**, 463 (1960).

upon treatment with Raney nickel, gave 2,6-diaminopurine[9] (III).

The synthesis of 2,6-dihydroxylaminopurine (II) and its 9-ribosyl derivative (VI) was achieved only by the reaction of ethanolic hydroxylamine and the corresponding 6-chloro-2-fluoro derivatives. Reaction of hydroxylamine with 2,6-dichloropurine led only to 2-chloro-6-hydroxylaminopurine, a reaction which is analogous to the conversion of 2,6-dichloropurine to 2-chloro-6-aminopurine upon aminolysis.[10] Apparently, the 6-methylthio is a better leaving group than the 6-chloro in 2-amino- or 2-hydroxypurines in their reactions with hydroxylamine and catalytic amounts of chloride ions.[11] However, when a fluorine atom is substituted at C_2, the methylthio group at C_6 is resistant toward nucleophilic displacement by hydroxylamine, and 2-hydroxylamino-6-methylthiopurine[12] is obtained even in the presence of chloride ions.

2-Fluoro-6-mercapto-9-β-D-ribofuranosylpurine[8] (IV) was chlorinated[13] to give 6-chloro-2-fluoro-9-β-D-ribofuranosylpurine (V) in 37% yield. Reaction of V with ethanolic hydroxylamine led to 2,6-dihydroxylamino-9-β-D-ribofuranosylpurine (VI), which was converted to the corresponding 2,6-diamino derivative[9b] (VII) by Raney nickel treatment. 2,6-Dihydroxylamino-9-β-D-ribofuranosylpurine (VI) was found to decompose in aqueous solution when kept at 5°. After a few weeks at room temperature, VI was transformed into a substance which showed a negative $FeCl_3$ test (loss of HONH group). Dihydroxylamino derivatives in the pyrimidine series have also been found to be unstable and readily oxidized,[14] as is dihydroxyurea.[15]

Solutions of V in ethanolic hydroxylamine (1 *M*) gave a positive $FeCl_3$ test (intense blue color) after a few minutes at 25°, indicative of a rapid reaction of hydroxylamine with V. This ease of reaction contrasts with the slow conversion rate of 6-chloro-, 2-amino-, and 2-hydroxy-6-chloropurines under the same conditions. 2-Fluoro-6-methylthio-9-β-D-ribofuranosylpurine (prepared from IV by methylation with CH_3I) failed to yield VI by hydroxylamine treatment, with or without chloride ions, and the starting material was recovered unchanged.

Biological Activity.—2,6-Dihydroxylaminopurine was evaluated, by methods previously reported,[16] for its chemotherapeutic activity in prolonging the survival time of mice with L1210 and P815 leukemias. This compound was also studied in sublines of L1210 leukemia resistant to 6-mercaptopurine (L1210/6-MP) and to 6-hydroxylamino-9-β-D-ribofuranosylpurine (L1210/HAPR), and in a line of P815 leukemia resistant to 6-mercaptopurine (P815/6-MP). These leukemias were carried in F_1 hybrids of the C57B1 × DBA/2 cross (BDF_1). Control and treatment groups contained ten mice each, and treatment was initiated 24 hr after the intraperitoneal inoculation of 1 million leukemic cells, and continued once daily intraperitoneally to a total of ten doses. Results are expressed in Table I as percent increased life span (ILS, %), values over 50% being considered significant, and values over 100% highly significant.

TABLE I

EFFECT OF 2,6-DIHYDROXYLAMINOPURINE (DHAP) (II) AND OTHER CHEMOTHERAPEUTIC AGENTS ON SURVIVAL TIME OF MICE WITH L1210 AND P815 LEUKEMIAS AND RESISTANT SUBLINES THEREOF

Leukemia	Drug	Dose, mg/kg/day × 10	Wt change, g/days[a]	Survival time, days	ILS, %[b]
L1210	DHAP	50	−1.2/8	13.2	59
	DHAP	25	+0.4/8	13.2	59
	6-MP[c]	20	−0.9/5	13.8	66
	Control		+2.6/5	8.3	
L1210/6-MP[d]	DHAP	100	+1.3/7	8.2	−15
	DHAP	50	+1.1/7	8.1	−16
	6-MP[c]	20	+2.2/7	9.3	−4
	HAPR[e]	200	+0.9/7	23.2	139
	Control		+3.4/7	9.1	
L1210/HAPR[f]	DHAP	50	−1.5/6	12.8	36
	DHAP	25	−0.3/6	13.7	46
	HAPR[e]	200	+1.6/6	8.2	−13
	6-MP[c]	20	−0.7/6	25.1	167
	Control		+1.9/6	9.4	
P815	DHAP	100	−2.3/11	10.3	16
	DHAP	50	+1.7/11	14.2	60
	6-MP[c]	20	+2.3/11	12.2	37
	Control		+2.5/4	8.9	
P815/6-MP[d]	DHAP	100	+1.0/8	8.0	−11
	DHAP	50	+1.1/8	8.5	−4
	6-MP[c]	20	+1.6/8	8.1	−9
	HAPR[e]	200	+0.4/8	21.6	142
	Control		+1.3/8	8.9	
L1210	HAPR[e]	200	+0.8/8	17.6	100
	Control		+4.0/8	8.5	

[a] Average weight change/day after injection on which given weight was taken. [b] Increase in life span (per cent). [c] 6-Mercaptopurine. [d] Resistant to 6-mercaptopurine. [e] 6-Hydroxylamino-9-β-D-ribofuranosylpurine. [f] Resistant to 6-hydroxylamino-9-β-D-ribofuranosylpurine.

2,6-Dihydroxylaminopurine was active against L1210 and P815 leukemias, the activity in L1210 leukemia being about the same to one-half that of 6-mercaptopurine on a molar basis. Sublines of L1210 and P815 leukemias resistant to 6-mercaptopurine were also resistant to 2,6-dihydroxylaminopurine. Since 6-mercaptopurine is known to be converted to an active nucleotide by IMP/GMP pyrophosphorylase, activity of which is reduced or lost in lines of mouse leukemia resistant to 6-mercaptopurine,[17] the cross-resistance in these resistant lines between 6-mercaptopurine and 2,6-dihydroxylaminopurine suggests that the latter compound may also be converted to an active nucleotide by a similar enzymatic pathway. A line of L1210 leukemia resistant to 6-hydroxylamino-9-β-D-ribofuranosylpurine (L1210/HAPR), which is probably converted to an active nucleotide by adenosine kinase,[3d] was still sensitive to 6-mercaptopurine and, to a lesser extent, to 2,6-dihydroxylaminopurine. In dosages that had a significant antileukemic effect in mice, there was no acute toxicity, as measured by weight loss, from 2,6-dihydroxylaminopurine.

(9) (a) A. Bendich, J. F. Tinker, and G. B. Brown, *J. Am. Chem. Soc.*, **70**, 3109 (1948); (b) J. Davoll and B. A. Lowy, *ibid.*, **73**, 1650 (1951).

(10) (a) J. A. Montgomery and L. Holum, *ibid.*, **79**, 2185 (1957); (b) S. R. Breshears, S. S. Wang, S. G. Bechtholt, and B. E. Christensen, *ibid.*, **81**, 3789 (1959).

(11) A. Giner-Sorolla, S. A. O'Bryant, J. H. Burchenal, and A. Bendich, *Biochemistry*, **5**, 3057 (1966).

(12) A. Giner-Sorolla, in preparation.

(13) (a) Wellcome Foundation Ltd., British Patent 767,216 (1957); *Chem. Abstr.*, **51**, 14796*d* (1957); (b) R. K. Robins, *J. Am. Chem. Soc.*, **82**, 2654 (1960); (c) J. F. Gerster, J. W. Jones, and R. K. Robins, *J. Org. Chem.*, **28**, 945 (1963); (d) R. K. Robins, *Biochem. Prepn.*, **10**, 145 (1963).

(14) (a) D. M. Brown and P. Schell, *J. Chem. Soc.*, 208 (1965); (b) I. Wempen, N. Miller, E. A. Falco, and J. J. Fox, in press.

(15) E. Boyland and R. Nery, *J. Chem. Soc.*, 360 (1966).

(16) M. R. Dollinger, J. H. Burchenal, W. Kreis, and J. J. Fox, *Biochem. Pharmacol.*, **16**, 689 (1967).

(17) R. W. Brockman, *Cancer Res.*, **25**, 1596 (1965).

2,6-Dihydroxylaminopurine was also evaluated against the Ridgeway osteogenic sarcoma grown in CHKRF (AKR/J × C3H) female mice. Fragments of solid tumor were inoculated subcutaneously *via* trochar and treatment was started, in groups of five mice, 5 days after tumor implantation and continued daily for 7 days. At a dosage of 62.5 mg/kg/day, the treated/control (T/C) value for average tumor diameter at the end of therapy was 0.52, and 1 week later was 0.35 (average of two experiments). These results are considered significant in this tumor system. Treatment with 31.3 mg/kg/day resulted in similar T/C values.

Screening tests with 2,6-dihydroxylamino-9-β-D-ribofuranosylpurine (VI) are being conducted and the results will be presented elsewhere.

Experimental Section[18]

2,6-Dihydroxylaminopurine (II).—6-Chloro-2-fluoropurine (I, 1.0 g, 5.5 mmoles) was dissolved in 1 *M* ethanolic NH_2OH (100 ml)[2b] containing 30% aqueous $NH_2OH \cdot HCl$ (1 ml)[11] and refluxed for 6 hr. After standing overnight at 25°, the resulting precipitate was washed (H_2O, EtOH) and dried. Colorless prisms were obtained which explode at 260° when preheated at 250°; yield 0.76 g (70%), recrystallized (80% aqueous EtOH). *Anal.* ($C_5H_6N_6O_2$) C, H, N.

Compound II gave an intense blue color with $FeCl_3$ solution and a red-brown color with 2 *N* NaOH, which darkened (azoxy formation). II exhibited at pH 7.0 (0.01 *M* phosphate buffer) λ_{max} 279 mμ (ε 8.0 × 10³), λ_{min} 261 mμ (ε 6.4 × 10³).

6-Chloro-2-fluoro-9-β-D-ribofuranosylpurine (V).—2-Fluoro-6-mercapto-9-β-D-ribofuranosylpurine[8] (IV, 5.6 g) (prepared from 2-amino-6-mercapto-9-β-D-ribofuranosylpurine[19]) was dissolved in concentrated HCl (7.5 ml) and MeOH (4 ml) and saturated at −5° with HCl. Cl_2 was bubbled through with mechanical stirring at −10° until a sample of the reaction mixture bleached pH indicator paper (45 min). The mixture was kept at −10° for 15 min and the pH was adjusted to 7.2 by careful addition of concentrated aqueous NH_3 at a temperature below 0°. The resulting slurry was dissolved in H_2O (*ca.* 50 ml) and extracted in a liquid–liquid extractor with ether for 48 hr. Upon evaporation of the ethereal extract, 2.1 g (37%) of colorless prisms, mp 158°, were obtained; recrystallized (EtOAc) mp 166°. *Anal.* ($C_9H_5ClFN_4O_4 \cdot 0.5H_2O$) C, H, Cl, F, N. V exhibited, at pH 1, λ_{max} 269 mμ (ε 9.6 × 10³); at pH 7.0 (0.01 *M* phosphate buffer), λ_{max} 269 mμ (ε 9.1 × 10³); at pH 13, λ_{max} 308 mμ (ε 7.7 × 10³), λ_{min} 269 mμ (ε 1.2 × 10³).

2,6-Dihydroxylamino-9-β-D-ribofuranosylpurine (VI).—A suspension of V (0.90 g, 3 mmoles) in 2 *M* anhydrous ethanolic NH_2OH (150 ml) was kept under N_2 at 25° for 15 min. After a few minutes at 25°, a positive $FeCl_3$ test (deep blue color) was obtained. The resulting solution was heated at 70° for 3 hr and evaporated to dryness *in vacuo* at room temperature to yield 0.95 g of a glass. A sample of this material was chromatographed on paper as a band using water saturated with BuOH. The uv-absorbing band was eluted with MeOH and a colorless crystalline material (short needles) was obtained, mp 190–192° (with charring). *Anal.* ($C_9H_{12}N_6O_6 \cdot 1.75H_2O$) C, H, N. VI gave an intense blue color with $FeCl_3$ solution and a red-brown color with 2 *N* NaOH, which darkened (azoxy formation). Aqueous solutions of VI decomposed after several days when kept at 5°. After a few weeks at 25°, VI (in solid form) was transformed into a substance which gave a negative $FeCl_3$ test (loss of HONH group). VI exhibited at pH 7.0 (0.01 *M* phosphate buffer), λ_{max} 222.5, 260, and 270 (shoulder), λ_{min} 243 mμ.

Refluxing 2-fluoro-6-methylthio-9-β-D-ribofuranosylpurine (1 g) with ethanolic NH_2OH (400 ml) in the presence (1 ml) or absence of 30% $NH_2OH \cdot HCl$ for 18 hr failed to yield VI. The starting material was recovered unchanged.

Treatment of II and VI with Raney Nickel.—2,6-Dihydroxylaminopurine (II, 29 mg) was suspended in a 5% aqueous NH_3 (5 ml) and Raney nickel (100 mg) was added. After boiling for 2 hr, the suspension was filtered while hot. The filtrate showed the uv spectrum of 2,6-diaminopurine[9] (III). Paper chromatograms in three solvent systems showed R_f values identical with those of an authentic sample of III. A similar treatment of VI resulted in its conversion into a solution with uv spectra at pH 6.7 and R_f values in three solvent systems identical with those of 2,6-diamino-9-β-D-ribofuranosylpurine[9b] (VII).

Hydrolysis of II and VI.[13,20]—II and VI were hydrolyzed with 2 *N* HCl (2 ml) at 100° for 1.5 min to xanthine which was identified by its uv spectrum at neutral, acid, and alkaline pH values.

Acknowledgment.—The authors wish to thank Dr. G. B. Brown for fruitful discussions. They are indebted to Dr. J. J. Fox and Miss I. Wempen (Sloan-Kettering Institute) and to Dr. H. Wood and Dr. R. B. Ing (National Institutes of Health) for generous gifts of thioguanosine. We thank Dr. G. Tarnowski of the Sloan-Kettering Institute for the screening data on 2,6-dihydroxylaminopurine on the Ridgeway osteogenic sarcoma.

(18) Ultraviolet absorption spectra were determined with a Cary recording spectrophotometer, Model 11. Paper chromatograms were run by the ascending method on Whatman No. 1 paper in the following solvent systems: water saturated with 1-butanol; 1-butanol saturated with water (with or without 10% ammonia); 1-butanol–formic acid–water (77:10:13, v/v). Melting points were taken in a Thomas–Hoover Unimelt apparatus and were corrected. The microanalyses were carried out by Spang Microanalytical Laboratory, Ann Arbor, Mich., and by Galbraith Analytical Laboratory, Knoxville, Tenn. Where analyses are indicated by the symbols of the elements only, analytical results obtained for those elements were within ±0.3% of the calculated values.

(19) J. J. Fox, I. Wempen, A. Hampton, and I. L. Doerr, *J. Am. Chem. Soc.*, **80**, 1669 (1958).

(20) Xanthine is also obtained by acid hydrolysis of isoguanine, its 1-N-hydroxy derivative and 2-hydroxy-6-hydroxylaminopurine (see ref 7 and J. C. Parham, J. Fissekis, and G. B. Brown, *J. Org. Chem.*, **32**, 1151 (1967)).