tially planar whereas the hydrophobic region near where the para position of the 6-anilino group is complexed appears to be nonplanar.

(3) Due to the differences in the hydrophobic bonding region of the enzyme in the 2 sources, several compds (**4**, **5**, **14**, **19**, **23**, **28**) show 500- to 900-fold better binding to the bacterial enzyme than the mammalian enzyme.

(4) The ability of the 4-EtO group of **17** to bind to the liver enzyme better than 4-C_4H_9-*n* of **18** or the 4-C_6H_5 of **19** due to a bend in the hydrophobic bonding area in this region is due to the greater conformational flexibility of the EtO group. If such is the case then higher alkyl or aralkyl groups may give highly potent inhibitors of the mammalian enzyme. Such studies are being pursued and already the most active inhibitor yet known has emerged; 6-(4-benzyloxy-2-methylanilino)-uracil has I_{50} = 14 μM and the $C_6H_5CH_2O$ substituent shows a 43-fold increment in binding over **9**.[33]

(5) Substituent effects on the 6-$C_6H_5(CH_2)_2NH$ (**33**), 6-$C_6H_5(CH_2)_4NH$ (**35**), 6-$C_6H_5O(CH_2)_3NH$ (**37**), and 5-$C_6H_5(CH_2)_4$ (**55**) uracils should be studied to determine how much binding can be enhanced.

(33) B. R. Baker and S. E. Hopkins, to be published.

Substituted Hydroxylaminopurines and Related Derivatives. Synthesis and Screening Tests[1]

ALFREDO GINER-SOROLLA* AND JOSEPH H. BURCHENAL

Divisions of Cell Biochemistry and Applied Therapy, Sloan-Kettering Institute for Cancer Research, New York, New York 10021

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Treatment of 6-chloro-2-fluoropurine (I) and its 9-β-D-ribosyl derivative (II) with NH_2OH at low temp yielded 2-fluoro-6-hydroxylaminopurine (III) and its 9-β-D-ribosyl derivative IV, resp. Compds III and IV were reduced to 2-fluoroadenine (VIII) and 2-fluoroadenosine (IX) with Raney Ni. The known 2-fluoro-6-mercaptopurine (X) was conveniently prepared from I and thiourea and transformed into 2-hydroxylamino-6-mercaptopurine (XI) with NH_2OH. I and methanolic NH_3 at 100° gave VIII or 2,6-diaminopurine (XVIII), depending on the duration of the treatment. I and X yielded, by reaction with the appropriate amino derivatives, several *N*-methylhydroxylamino-, -methoxyamino-, and -methylaminopurines. Reaction of I with hydrazine gave 2-fluoro-6-hydrazinopurine (XXI). Equimolar amounts of I and XXI afforded 6,6'-bis(2-fluoroadenine) (XXII) which on Raney Ni treatment was reduced to VIII. Interaction of VIII with NH_2OH resulted in the synthesis of 2-hydroxylaminoadenine (XXIII). Reaction of 2-fluoropurine (XXIV) with NH_2OH yielded 2-hydroxylaminopurine (XXV). III and XI possessed inhibitory activity against P815 mouse leukemia, and X caused marked reduction of Ridgeway osteogenic sarcoma; IV was inactive.

New substituted purines have been prepared in continuation of studies of biologically active purine derivatives.

The synthesis of 2-fluoro-6-hydroxylaminopurine and its 9-β-D-ribofuranosyl derivative was accomplished in order to study the effect of the F atom at C_2 on the growth-inhibitory effect and toxicity of 6-hydroxylaminopurine[2] and of its 9-β-D-ribofuranosyl derivative.[3] Similarly, 2-hydroxylamino-6-mercaptopurine was prepared in order to investigate the influence of the HONH group at the C_2 of 6-mercaptopurine;[4] this compd may also be considered as the 2-*N*-OH derivative of thioguanine.[5] Several substituted *N*-methylhydroxylamino, methoxyamino, *N*-methylamino, hydrazino, and 6,6-bis(2-fluoroadenine) derivatives were synthesized as well as the 2-*N*-hydroxylamino derivative of adenine, 2-hydroxylamino-6-aminopurine, to study their potential growth-inhibitory activity.

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(2) (a) A. Giner-Sorolla and A. Bendich, *J. Amer. Chem. Soc.*, **80**, 3932 (1958); (b) A. Giner-Sorolla, Ph.D. Thesis, Cornell University, Ithaca, N. Y., 1958; *Diss. 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*, **19**, 97 (1966).

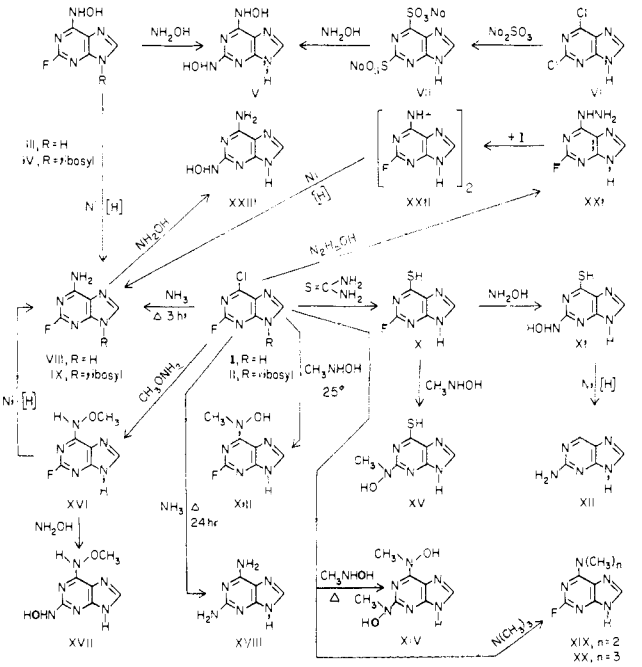
(3) (a) A. Giner-Sorolla, L. Medrek, and A. Bendich, *J. Med. Chem.*, **9**, 143 (1966); (b) J. H. Burchenal, M. Dollinger, J. Butterbaugh, D. Stoll, and A. Giner-Sorolla, *Biochem. Pharmacol.*, **16**, 423 (1967).

(4) G. B. Elion, E. Burgi, and G. H. Hitchings, *J. Amer. Chem. Soc.*, **74**, 411 (1952).

(5) G. B. Elion and G. H. Hitchings, *ibid.*, **77**, 1676 (1955).

Synthetic Studies.—Treatment of 6-chloro-2-fluoropurine (I)[6] (Scheme I) with an excess of ethanolic

SCHEME I



$HONH_2$ at 5° afforded 2-fluoro-6-hydroxylaminopurine (III) in 83% yield. A minor by-product, 2,6-dihydroxylaminopurine (V),[7] was also obtained. When

(6) J. A. Montgomery and K. Hewson, *ibid.*, **82**, 463 (1960).

(7) A. Giner-Sorolla, C. Nanos, J. H. Burchenal, M. Dollinger, and A. Bendich, *J. Med. Chem.*, **11**, 52 (1968).

III was treated with refluxing ethanolic $HONH_2$, complete transformation to V occurred. Similarly, 9-β-D-ribofuranosyl-6-chloro-2-fluoropurine (II)[7] with $HONH_2$ at −5° gave 9-β-D-ribofuranosyl-2-fluoro-6-hydroxylaminopurine (IV) which proved to be very unstable.

A more convenient synthesis of 2,6-dihydroxylaminopurine (V) resulted from the ethanolic $HONH_2$ treatment of purine-2,6-disulfonate (VII), which, in turn, was prepared from 2,6-dichloropurine[8] and Na_2SO_3.

Recently, the synthesis of the 2′,3′,5′-triacetyl derivative of IV has been described; an unstable product[9] resulted from its deacetylation. Compounds III and IV were reduced with Raney Ni to the known 2-fluoroadenine[6] (VIII) and 2-fluoroadenosine[6] (IX), resp.

When 6-chloro-2-fluoropurine (I) was heated with an excess of methanolic NH_3 at 100° for 3 hr, 2-fluoroadenine (VIII) was formed. When the ammonolysis was prolonged to 24 hr at 100°, I was completely transformed into 2,6-diaminopurine[10,11] (XVIII).

The known 2-fluoro-6-mercaptopurine[6] (X) was readily prepared by reaction of 6-chloro-2-fluoropurine (I) with thiourea.[12] Interaction of X with ethanolic $HONH_2$ at 25° or brief refluxing gave 2-hydroxylamino-6-mercaptopurine (XI) which upon treatment with Raney Ni was converted into 2-aminopurine[13] (XII). Compound X and HONHMe yielded 2-methylhydroxylamino-6-mercaptopurine (XV). Reaction of I at 25° with HONHMe proceeds almost instantly to yield 2-fluoro-6-*N*-methylhydroxylaminopurine (XIII) and 2,6-bis(*N*-methylhydroxylamino)purine (XIV) after brief refluxing with the same reagent. Reaction of I with $MeONH_2$ afforded 2-fluoro-6-methoxyaminopurine (XVI), which with $HONH_2$ gave 2-hydroxylamino-6-methoxyaminopurine (XVII). I was transformed with NMe_3 into a mixture of 2-fluoro-6-trimethylaminopurine betaine (XIX) and the known[6] 2-fluoro-6-dimethylaminopurine (XX).

When I was treated with cold ethanolic hydrazine 2-fluoro-6-hydrazinopurine (XXI) was obtained. Equimolar amounts of I and XXI yielded 6,6′-bis(2-fluoroadenine) (XXII). Compounds XVI, XXI, and XXII were transformed with Raney Ni into 2-fluoroadenine (VIII). Treatment of VIII with $HONH_2$ resulted in the synthesis of 2-hydroxylaminoadenine (XXIII), which was transformed into 2,6-diaminopurine (XVIII) with ethanolic hydrazine. Refluxing of X with Raney Ni gave the known[6] 2-fluoropurine (XXIV) which was transformed to 2-hydroxylaminopurine (XXV) with $HONH_2$.

Screening Tests.—The screening technique for the evaluation of drugs by their ability to prolong the survival time of mice with transplanted leukemia has been reported previously.[14,3b]

2,6-Dihydroxylaminopurine[7] had the unique property of exerting a greater inhibitory activity against mouse leukemia P815 at doses of 3 mg/kg (qd for 5 days) (average survival time 26 days *vs.* 9 days' control) than at 0.6, 1.5, 12.5, 25, or 50 mg, it was toxic at 200 mg.[15] 2-Hydroxylamino-6-mercaptopurine (XI) showed a similar property and was most active in the same mouse leukemia and dosage schedule at 1.25 mg (average prolongation of survival time *ca.* 22 days *vs.* 9 days of controls), while it was inactive at 0.6 mg (survival *ca.* 10 days) and less effective at 2.5 mg (survival *ca.* 17 days) and at 5 mg (survival *ca.* 14 days).

2-Fluoro-6-hydroxylaminopurine (III) was less effective than XI and it did not show a peak of activity at low dosage: at 12.5 mg/kg (qd for 5 days) the average survival was 11 days (*vs.* 9 days for controls) at 25 mg it was 14 days and at 50 mg was toxic. The 9-β-D-ribofuranosyl derivative (IV) was devoid of antileukemic activity, presumably due to its instability in aq solns.

In Ridgeway osteogenic sarcoma, 2-fluoro-6-mercaptopurine (X) at 400 mg/kg (qd for 6 days) produced a marked reduction of tumor diameter, but it also showed toxicity. Screening tests with 2-hydroxylaminopurine (XXV) in L1210 mouse leukemia were negative. This compd is the *N*-OH analog of the potent mutagen 2-aminopurine.[16] In tests with phage T4 it possessed no mutagenic effects.[17]

Experimental Section[18]

2-Fluoro-6-hydroxylaminopurine (III).—A soln of 6-chloro-2-fluoropurine[6,19] (I, 4.0 g, 0.023 mole) in 0.6 *M* ethanolic $HONH_2$ (1500 ml) was kept at 5° for 48 hr. The small ppt of crude 2,6-dihydroxylaminopurine[7] (V) (0.2 g) which formed was removed by filtration, and the filtrate was concd to about 50 ml *in vacuo* below 25°. The resulting suspension was collected by filtration, washed thoroughly with cold H_2O and EtOH to yield 3.26 g (83%) of a white material, mp 270° (dec when inserted at 260°). An anal. sample was prepd by acidification of a suspension of III in H_2O with 2 *N* HCl, charcoal treatment, and neutralizing the filtrate with solid NaOAc. After repeated washing with cold H_2O, colorless needles were obtd: mp 270° dec; uv_{max} (pH 6) (0.1 *M* adipic acid, 0.1 *M* KOH, 80:140, v/v) 271 nm (ϵ 14.9 × 10^3); pK_a = 2.42 (±0.07). Instability at other values of pH did not allow further uv determinations; solubility in H_2O at 25° (±1°) was 0.48 g/l. *Anal.* ($C_5H_4N_5FO$) C, H, N, F.

2-Fluoro-6-hydroxylaminopurine (III) gave with 2 *N* NaOH a brown soln which rapidly turned black. A suspension of III in H_2O decompd upon boiling. When a suspension of III (10 mg) in H_2O (5 ml) was refluxed with Raney Ni (50 mg) for 30 min, a soln with uv spectra and R_f values identical with those of 2-fluoroadenine[6] (V) was obtd.

9-β-D-Ribofuranosyl-2-fluoro-6-hydroxylaminopurine (IV).—A soln of 9-β-D-ribofuranosyl-6-chloro-2-fluoropurine hemihydrate[7] (II, 1.0 g, 3.2 mmoles) in 0.6 *M* ethanolic $HONH_2$ (75 ml) was kept at −5° for 45 min. The resulting ppt was collected, washed with Et_2O, and immediately dried *in vacuo* over P_2O_5 to yield 0.76 g of a pale yellow product which turned gummy on exposure to air: uv_{max} (pH 5.5) (H_2O) 270 and 293 (shoulder), (pH 13) (0.1 *N* NaOH) 297 nm. Instability of the

(8) J. A. Montgomery and L. B. Holum, *J. Amer. Chem. Soc.*, **80**, 404 (1958).

(9) J. S. Montgomery and K. Hewson, *J. Med. Chem.*, **13**, 427 (1970).

(10) *Cf.* (a) A. Bendich, J. F. Tinker, and G. B. Brown, *J. Amer. Chem. Soc.*, **70**, 3109 (1948). (b) G. B. Brown and V. S. Weliky, *J. Org. Chem.*, **23**, 125 (1958).

(11) W. Traube, *Ber.*, **37**, 4547 (1904).

(12) Cf. A. Bendich, P. J. Russell, Jr., and J. J. Fox, *J. Amer. Chem. Soc.*, **76**, 6072 (1954), for the thiation of 6-chloropurine.

(13) S. F. Mason, *J. Chem. Soc.*, 2071 (1954).

(14) J. H. Burchenal, J. R. Burchenal, M. N. Kushida, S. F. Johnston, and B. S. Williams, *Cancer*, **2**, 113 (1949).

(15) J. H. Burchenal, unpublished experiments, and data courtesy of Dr. H. B. Wood, Jr., C.C.N.S.C., National Cancer Institute, Bethesda, Md.

(16) (a) E. Freese, *J. Mol. Biol.*, **1**, 87 (1959); (b) E. Freese, *Angew. Chem., Int. Ed. Engl.*, **8**, 12 (1969).

(17) E. Freese, personal communication.

(18) Uv spectra were determined with a Cary recording spectrophotometer, Model 11. Ascending paper chromatography was run on Whatman No. 1 paper in the following solvent systems: concd aq NH_4–H_2O–*i*-PrOH (10:20:70); 1-BuOH–H_2O–AcOH (50:25:25); and 1 *M* NH_4OAc–EtOH (30:70). The detn of mp's points was carried out with a Mel-Temp melting point apparatus and the temps were corrected. Analyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Mich. When anal. are indicated by symbols of the elements or functions, anal. results obtained for those elements or functions were within ±0.4% of the theor values.

(19) The yield of pure I by the Montgomery[6] method prepn from batches of 15 g of 2-amino-6-chloropurine was increased to 30% by using 25% less HBF_4, prolonged Et_2O extn (72 hr) and repeated charcoal treatment of the mother liquors of crystn.

compd did not allow ϵ detns. *Anal.* ($C_{10}H_{12}N_5FO \cdot 1.75H_2O$) C, H, N. Qual test for F was pos.

IV gave a strong violet color with $FeCl_3$ and a dark brown soln with 2 *N* NaOH (azoxy formation). When 10 mg of this material was boiled in H_2O (5 ml) with Raney Ni (50 mg) for 30 min, the supernatant showed uv spectra at 3 pH values identical with those of 2-fluoroadenosine.[6]

2-Fluoro-6-mercaptopurine (X).—Thiourea (0.50 g, 6.6 moles) was added to a soln of 6-chloro-2-fluoropurine[6] (I, 1.03 g, 6 mmoles) in EtOH (10 ml). After 10-min refluxing, a copious ppt appeared. The mixt was stirred and refluxed for a total of 2 hr and cooled, and the ppt was collected and washed with EtOH to yield 0.95 g (93%) of yellow prisms, mp > 300°. This product was identical with a sample of 2-fluoro-6-mercaptopurine (X)[6] prepd from thioguanine.

Reaction of X with Raney Ni.—A suspension of 2-fluoro-6-mercaptopurine (X, 0.60 g, 3.5 mmoles) in H_2O (50 ml) and Raney Ni (1.5 g) was boiled for 3 hr. The mixt was filtered through a Celite pad when hot, the ppt was washed twice with boiling H_2O, and the combined filtrates were evapd to dryness *in vacuo*. The residue was washed with a little EtOH to yield 73 mg of crude 2-fluoropurine[6] (XXIV) that was used without further purification to prep 2-hydroxylaminopurine (XXV).

2-Hydroxylamino-6-mercaptopurine Hydrate (XI).—A suspension of 2-fluoro-6-mercaptopurine (X, 0.50 g, 2.9 mmoles) in 0.6 *M* ethanolic $HONH_2$ (200 ml) was stirred at 25°. Soln occurred after 1 hr; the soln was treated with charcoal, filtered, and kept at 0° for 48 hr. It was again treated with charcoal, filtered, and evapd *in vacuo* below 20°. The resulting syrup was suspended in H_2O (10 ml) and cooled, and the pH was adjusted to 5 by careful add of glacial AcOH. The resulting ppt was washed with cold H_2O and dried to yield short yellow prisms, 0.32 g (58%). An anal. sample was prepd by repeated washing with H_2O and EtOH: mp 260° (dec when inserted at 255°); uv_{max} (pH 2) (0.01 *N* HCl) 214 (ϵ 19.3 × 10^3) and 340 nm (18.0 × 10^3), (pH 5) (0.1 *M* AcOH, 0.1 *M* NaOH) 217 (19.1 × 10^3) and 342 (19.9 × 10^3), (pH 9) (0.025 *M* $Na_2B_4O_7$ and 0.01 *M* $B(OH)_3$) 217 (16.5 × 10^3) and 318 (15.3 × 10^3). *Anal.* ($C_5H_5N_5OS \cdot H_2O$) C, H, N, S. The solubility of XI at 25° (±1°) was 0.27 g/l.

XI gave a dark violet soln with $FeCl_3$ (HONH function) and a dark brown soln with 2 *N* NaOH which later yielded a dark orange cryst ppt (azoxy formation). A suspension of XI (20 mg) in H_2O (5 ml) and Raney Ni (100 mg) when boiled for 1 hr gave a soln with uv spectra and R_f values identical with those of an authentic sample of 2-aminopurine[13] (XII).

XI could also be obtained in comparable yield from X by brief refluxing (up to 1 hr) in ethanolic NH_2OH. Prolonged refluxing (18 hr), however, yielded 2-hydroxylamino-6-hydroxypurine[20] by hydrolytic cleavage.

Reaction of 6-Chloro-2-fluoropurine (I) with Methanolic NH_3. A. Brief Treatment.—A soln of 6-chloro-2-fluoropurine (I, 0.5 g, 3 mmoles) in MeOH satd with NH_3 (25 ml) was heated in a glass-lined, high-pressure cylinder at 100° for 3 hr. The mixt was cooled and evapd to dryness *in vacuo*, and the residue was taken up in 70% aq EtOH and collected. Upon fraction crystns from 70% aq EtOH, 65 mg (14%) of chromatographically homogeneous 2-fluoroadenine[6] (VIII) was obtd.

B. Prolonged Treatment.—A soln of 6-chloro-2-fluoropurine (I, 0.5 g, 3 mmoles) in a satd methanolic NH_3 (20 ml) was heated as above at 100° for 24 hr. After cooling, the resulting suspension was evaporated to dryness under reduced pressure and washed with 70% aq EtOH to yield 0.31 g (71%) of crude 2,6-diaminopurine (XVIII).

2,6-Dihydroxylaminopurine (V). Method A. From Purine-2,6-disulfonate (VII).—A soln of purine-2,6-disulfonate disodium salt[21] (IX, 5.2 g, 16 mmoles) in H_2O (200 ml) was added to 0.6 *M* ethanolic $HONH_2$ (1300 ml), and the mixt was refluxed for 5 hr and kept at 25° overnight. The ppt was collected, washed with H_2O, and dried to give 1.8 g of a white solid, mp 260° (dec when inserted at 250°). From the filtrate upon concn *in vacuo* a second batch was obtained (0.6 g, mp 260° dec, yield, 2.4 g, 82%). This compd was identical with that previously prepd[7] from 6-chloro-2-fluoropurine.

Method B. From 2-Fluoro-6-hydroxylaminopurine (III).—A suspension of 2-fluoro-6-hydroxylaminopurine (III, 100 mg, 0.6 mmole) in 1 *M* ethanolic $HONH_2$ (100 ml) was refluxed for 6 hr. After cooling, the resulting ppt was collected, washed with cold H_2O, and dried to yield 84 mg (82%) of 2,6-dihydroxylaminopurine[7] (V), mp 260° (dec when inserted at 250°).

2-Fluoro-6-*N*-methylhydroxylaminopurine (XIII).—6-Chloro-2-fluoropurine (I, 1.72 g, 19 mmoles) in EtOH (15 ml) was added at 25° to a soln of MeNHOH [prepd from solns of MeNHOH·HCl (8.35 g, 0.1 mole) in EtOH (75 ml) and KOH (*ca.* 5.5 g) in EtOH (25 ml), filtration of the KCl formed, and treatment with charcoal]. A thick cryst mass rapidly formed. After stirring at 25° for 1 hr, the ppt was collected, washed with cold H_2O and EtOH, and dried *in vacuo* over P_2O_5 to yield 1.54 g (84%) of colorless thin fibrous crystals: mp 242° dec; uv_{max} (pH 0) 212 (ϵ 13.6 × 10^3) and 281 (14.1 × 10^3), (pH 5.0) 282 (ϵ 14.5 × 10^3), (pH 12) 307 nm (11.7 × 10^3). *Anal.* ($C_6H_8N_5OF$) C, H, N, F.

Aq solns of XIII gave a light blue color with $FeCl_3$, and orange-brown with 2 *N* NaOH.

2,6-Bis(*N*-methylhydroxylamino)purine (XIV).—6-Chloro-2-fluoropurine (I, 1.72 g, 8.5 mmoles) in EtOH (15 ml) was added to a soln of MeNHOH (prepd as above) and refluxed for 3 hr. The abundant cryst ppt which appeared redissolved on further heating, and later a new ppt was formed. The product was collected by filtration, washed repeatedly with cold H_2O, and dried to yield 1.66 g (79%) of a cryst material: mp 270° dec; uv_{max} (pH 3) 264 (ϵ 16.6 × 10^3), 281 (shoulder) (14.5 × 10^3), (pH 7) 292, (pH 12) 308 nm; instability of neutral and alkaline pH did not allow ϵ detn. *Anal.* ($C_6H_{10}N_6O_2$) C, H, N.

2,6-Bis(*N*-methylhydroxylamino)purine (XIV) rapidly turned purple when exposed to light; its aq suspensions gave a strong violet color with $FeCl_3$ and a deep brown solution with 2 *N* NaOH which deposited a thick cryst mass after a few min.

2-*N*-Methylhydroxylamino-6-mercaptopurine (XV).—A soln of 2-fluoro-6-mercaptopurine (X, 1.69 g, 10 mmoles) in ethanolic MeNHOH (100 ml) was kept at 5° for 24 hr. The soln was filtered through a Celite–charcoal pad and evapd to a syrup under reduced pressure, EtOH was added, and the resulting cryst ppt was collected by filtration and repeatedly washed with EtOH. After drying *in vacuo* over P_2O_5, 1.1 g (56%) of a yellow cryst product was obtained: mp 240° (explodes when inserted at 230°); uv_{max} (pH 1.0) 215 (ϵ 21.8 × 10^3), 268 (ϵ 10.9 × 10^3), 351 (ϵ 16.2 × 10^3), (pH 5.0) 220 (ϵ 19.2 × 10^3), 267 (ϵ 8.9 × 10^3), 344 (ϵ 17.8 × 10^3), (pH 12.0) 236 (ϵ 11.9 × 10^3), 310 nm (ϵ 14.6 × 10^3). *Anal.* ($C_6H_7N_5OS$) C, H, N, S.

2-Fluoro-6-methoxyaminopurine (XVI).—A soln of 6-chloro-2-fluoropurine (I, 7.0 g, 0.04 mole) in MeOH (200 ml) was added to a soln of $MeONH_2$ [prepd from 42.5 g of $MeONH_2 \cdot HCl$ (0.5 mole) in MeOH (150 ml) and sufficient 10% ethanolic KOH to bring the pH to 9]. The mixt was refluxed for 3 hr and kept at 25° for 24 hr. The ppt which resulted was collected, repeatedly washed with cold H_2O, and dried to yield 5.5 g (73%) of thin threads: mp > 300°, uv_{max} (pH 0) 212 (13.6 × 10^3), 281 (14.1 × 10^3), (pH 5) 282 (14.5 × 10^3), (pH 12) 307 nm (11.7 × 10^3). *Anal.* ($C_6H_6N_5FO$) C, H, N, F. Reaction of I with $MeONH_2$ at 0° gave a lower yield of XVI.

Treatment of XVI with Raney Ni.—A suspension of 2-fluoro-6-methoxyaminopurine (XVI, 5.5 g, 0.03 mole) in H_2O (800 ml) and Raney Ni (24 g) was refluxed for 6 hr. The suspension was filtered when hot, the Ni was washed twice with boiling H_2O, and the combined filtrates were evapd *in vacuo*. After cooling the soln overnight at 5°, the resulting ppt was collected by filtration, and washed with H_2O and EtOH to yield 1.9 g of a product identical with 2-fluoroadenine (VIII). It was used without further purification for the synthesis of 2-hydroxylaminoadenine (XXIII).

2-Hydroxylamino-6-methoxyaminopurine (XVII).—A soln of 2-fluoro-6-methoxyaminopurine (XVI, 0.56 g, 3 mmoles) in 0.6 *M* ethanolic $HONH_2$ (400 ml) was refluxed for 5 hr. The reaction mixt was evapd to dryness under reduced pressure and the residue washed with a little cold H_2O, and dried to yield 0.39 g (65%) of colorless crystals; mp 240° dec; uv_{max} (pH 1.0) 254 (ϵ 1.6 × 10^3), 282 nm (10.5 × 10^3), (pH 5.0) 281, (pH 13.0) 226, 281 nm. Unstable at pH 5 and above. *Anal.* ($C_6H_8N_6O_2$) C, H, N.

Reaction of 6-Chloro-2-fluoropurine (I) with Me_3N.—A soln

(20) A. Giner-Sorolla, S. A. O'Bryant, C. Nanos, M. R. Dollinger, A. Bendich and J. H. Burchenal, *J. Med. Chem.*, **11**, 521 (1968).

(21) Purine-2,6-disulfonate, disodium salt, was prepd by heating 2,6-dichloropurine (4.3 g, 23 mmoles) in H_2O (40 ml) and Na_2SO_3 (7 g, 55 mmoles) at 70° for 3 hr. After cooling, the soln was poured in 500 ml of EtOH, and the resulting white ppt was collected by filtration: yield, 5.2 g (84%). *Anal.* ($C_5H_2N_4O_6Na_2S_2$) C, H, N; S: calcd, 18.74; found, 17.63. *Cf.* G. Dryhurst, *J. Electrochem. Soc.*, **117**, 1113 (1970), for 2 other methods of prepn of purine-2,6-disulfonate, K salt.

of 6-chloro-2-fluoropurine (I, 1.5 g, 8.6 mmoles) in 25% methanolic Me_3N was kept at 25°. After a few min a cryst ppt appeared. The mixt was kept at 25° for 48 hr; the ppt was collected and washed with EtOH to yield colorless plates (0.45 g, 26%), mp 300°, of 2-fluoro-6-trimethylaminopurine betaine (XX): uv_{max} (pH 1.0) 270.5 (ϵ 7.9 × 10^3), (pH 6) 273 (7.3 × 10^3), (pH 12) 273 nm (7.6 × 10^3). *Anal.* ($C_8H_{11}N_5F$) C, H, N, F.

The filtrate of the above reaction, after evapn to dryness under reduced pressure, gave a cryst residue consisting of 2-fluoro-6-dimethylaminopurine (XIX), 1.1 g (70%), mp 220°. *Anal.* ($C_7H_8N_5F$) C, H, N, F. This material was identical with the product prepared by Montgomery and Hewson.[6]

Reaction of 2-Fluoro-6-chloropurine (I) with Hydrazine.—A 10% hydrazine hydrate ethanolic soln (25 ml) was added to 2-fluoro-6-chloropurine (I, 1.5 g, 8.7 mmoles) dissolved in EtOH (25 ml) at 5°. After stirring at 5° for 1 hr, the resulting ppt was collected by filtration, and dried to yield 2-fluoro-6-hydrazinopurine (XXI), 1.3 g (87%) of thin needles, mp 142°. *Anal.* ($C_5H_6N_6F$) C, H, N, F.

When XXI (20 mg) was boiled in H_2O (5 ml) and Raney Ni (50 mg) for 2 hr, the resulting soln showed uv spectra and R_f values identical with those of 2-fluoroadenine (VIII).

6,6-Bis(2-fluoroadenine) (XXII).—Solns of 2-fluoro-6-chloropurine (I, 1.20 g, 6.9 mmoles) in EtOH (25 ml) and 2-fluoro-6-hydrazinopurine (XXI, 1.15 g, 6.9 mmoles) in 70% aq EtOH (25 ml) were combined. Anhyd NaOAc (0.67 g, 7.6 mmoles) was added, and the mixt was refluxed for 6 hr and kept at 25° overnight. The ppt which formed was collected by filtration and repeatedly washed with H_2O and EtOH to yield 1.03 g (quant) of a yellow microcryst product, mp >300°. *Anal.* ($C_{10}H_6N_{10}F_2$) C, H, N. F test was positive.

Treatment of XXII with Raney Ni.—A suspension of XXII (0.5 g, 1.6 mmoles) in H_2O (25 ml) and Raney Ni (3 g) was refluxed for 12 hr. The reaction mix was filtered when hot, the Ni was washed with boiling H_2O, and the combined filtrates were evapd to dryness under reduced pressure. The residue was suspended in H_2O (5 ml), filtered, and dried to yield 70 mg (14%) of 2-fluoroadenine (VIII).

2-Hydroxylamino-6-aminopurine (2-Hydroxylaminoadenine) (XXIII).—A suspension of VIII (0.30 g, 1.8 mmoles) in 0.6 *M* ethanolic $HONH_2$ (300 ml) and 0.3 ml of 30% aq soln of $HONH_2 \cdot HCl$[22] was refluxed for 6 hr and kept at 25° overnight. The resulting ppt was collected by filtration, washed with H_2O, and dried to yield 0.17 g (53%) of microneedles: mp 270° dec; uv_{max} (pH 3.0) 242 (shoulder) (ϵ 12.2 × 10^3), 280 (10.1 × 10^3), (pH 7.0) 253 (8.6 × 10^3), 274 (9.1 × 10^3); pK_a = 4.74 (±0.04). *Anal.* ($C_5H_6N_6O \cdot 0.33H_2O$) C, H, N. XXXIII gave blue-green color with $FeCl_3$ soln (HONH) and deep orange-brown color with 1 *N* NaOH (azoxy formation). Boiling of XXIII with 5% ethanolic hydrazine for 3 hr gave a soln with uv spectra and R_f values identical with those of 2,6-diaminopurine (XVIII).

2-Hydroxylaminopurine (XXV).—A soln of 2-fluoropurine[6] (XXIV, 1.2 g, 8.5 mmoles) in 1 *M* ethanolic $HONH_2$ was refluxed for 10 hr and kept at 25° overnight. The resulting ppt was collected and washed with cold H_2O to yield a pale yellow cryst material, 0.83 g, mp 260° (exploded when inserted at 250°). Upon concn of the filtrate to about 30 ml, a second crop (0.31 g, mp 260°, expl) was obtd (yield, 87%). An anal. sample was obtd by thorough washing of the first ppt with 90% aq MeOH at 25°: uv_{max} (pH 7.0) 233 (ϵ 8.6 × 10^3), 346 nm (13.6 × 10^3); pK_{a_1} = 2.08 (±0.05), pK_{a_2} = 8.52 (±0.1). *Anal.* ($C_5H_4N_5O \cdot 0.33H_2O$) C, H, N. A suspension of XXV gave an intense dark blue color with $FeCl_3$ soln. When XXV was dissolved in 2 *N* NaOH an orange soln was obtd, but in contrast to 6-hydroxylaminopurine,[2] no ppt of the corresponding azoxy derivative was observed. The uv spectra of this soln showed profound decompn. A sample of XXV (10 mg) was dissolved in 5% aq NH_3 (5 ml) and Raney Ni (50 mg) was added. After boiling for 15 min the resulting soln showed uv spectra and R_f values identical with those of 2-aminopurine.[13]

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Xylo- and Arabinofuranosylthioguanine and Related Nucleosides Derived from 2-Acetamido-6-chloropurine[1]

WILLIAM W. LEE,* ABELARDO P. MARTINEZ, ROBERT W. BLACKFORD, VICTOR J. BARTUSKA, ELMER J. REIST, AND LEON GOODMAN[1]

Life Sciences Research, Stanford Research Institute, Menlo Park, California 94025

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9-(β-D-Xylo- and 9-(α- and β-D-arabinofuranosyl)thioguanine (**1**, **α-5**, and **β-5**) have been synthesized. The Hg derivative of 2-acetamido-6-chloropurine gave 9-(2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl)- and 9-(2,3,5-tri-*O*-acetyl-β-D-xylofuranosyl)-2-acetamido-6-chloro-9*H*-purine (**9** and **10**, respectively) on reaction with the appropriate halo sugar. Treatment of **9** and **10** with NaSH and deacylation gave **α-5** and **1**, respectively. Compd **10** was converted to the xyloside of 2-amino-6-chloropurine (**11**) and guanine (**2**). Both of these could be converted through several intermediates to the 2',3'-anhydronucleoside intermediate **15g**. Cleavage with NaOAc in aq DMF afforded 9-(β-D-arabinofuranosyl)guanine (**β-6**). Appropriate acylation, followed by thiation and deacylation, gave **β-5**, which was active against leukemia L1210 in mice; the other nucleosides tested were inactive.

Many compounds with antitumor activity have been found among purines and nucleosides. Thioguanine and thioguanosine, for example, are useful carcinostatic agents.[2] Certain thioguanine nucleosides synthesized in these laboratories—*e.g.*, 3'-deoxythioguano-

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